REMARKS

Claims 29-43 are pending. Claims 1-28 were cancelled. Claims 29-43 were previously presented.

Reconsideration of the application in view of the remarks provided below is respectfully requested.

§ 103 Rejections

The Examiner rejected Claims 29-43 under 35 U.S.C. § 103(a) as being unpatentable over US Patent 5,141,858 ("Paul") in view of US Patent 5,702,942 ("Leathers"). Applicants respectfully traverse this rejection.

The present invention relates to low-glycemic sweeteners. A sweetener which can be termed low-glycemic is one which has a low glycemic index. Glycemic index is not a measure of the caloric value of a sweetener. Rather, the glycemic index (a.k.a. glycaemic index) is a measure of how rapidly and to what extent a carbohydrate breaks down during digestion and releases glucose into the bloodstream. Thus, carbohydrates which break down rapidly and release glucose into the bloodstream quickly during digestion are labeled "high-glycemic", whereas carbohydrates which break down slowly and release glucose into the bloodstream gradually are labeled "low-glycemic". Carbohydrates which do not release glucose into the bloodstream during digestion do not have a glycemic index at all and therefore may not be characterized as either "high-glycemic" or "low-glycemic". Accordingly, a non-digestible fiber would not have a glycemic index at all because glycemic index is a measure of the rate and extent of glucose rise in the blood.

Glyemic index is a representation of a carbohydrate's standardized ability to increase a subject's blood glucose level relative to an equivalent dose of a standard carbohydrate. While

this comparison could be done in a number of ways, one method is described in *The Glycaemic Index: A Physiological Classification of Dietary Carbohydrate*, by T.M.S. Wolever. This method is briefly described below, and the relevant chapter of this book is attached with this Office Action Response for Examiner's reference.

In the attached reference, glycemic index is defined as "the incremental area under the blood glucose response curve elicited by a 50 g available carbohydrate portion of a food expressed as a percentage of the response after 50 g anhydrous glucose taken by the same subject." (T.M.S. Wolever, *The Glycaemic Index: A Physiological Classification of Dietary Carbohydrate*, 2006; p. 11). Blood glucose response curves are obtained by testing the blood glucose of subjects who have consumed 50 g available carbohydrate portion of a food. (*Id.* at p. 17) Blood samples are taken from these subjects by finger prick at 15, 30, 45, 60, 90, and 120 minutes after the subject has started to eat a test meal consisting of 50 g available carbohydrate portion of a food. (*Id.*) The blood glucose values obtained are plotted on a graph having "blood glucose" on the Y-axis and "time" on the X-axis. The same procedure is conducted with the same subject for 50 g of the reference food – anhydrous glucose. (*Id.* at p. 18) The area under both curves is determined and the glycemic index of the available carbohydrate tested is expressed a percentage: the area under its curve divided by the area under the curve obtained for anhydrous glucose.

Importantly, the glycemic index is "an index of the extent to which the <u>available carbohydrate</u> in the food raises the blood glucose." (*Id.* at p. 14; emphasis added) "Therefore, unavailable, or non-glycaemic, carbohydrates should be excluded ... because, by definition, these carbohydrates do not raise blood glucose." (*Id.* at p. 16) Only carbohydrates which will release glucose into the bloodstream have a "glycemic index". It is therefore improper to label a carbohydrate which does not release glucose into the bloodstream as either high-glycemic or low-glycemic. These unavailable carbohydrates simply do not have a glycemic index.

The United States is facing a significant increase in the incidence of diabetes in its populace. Indeed, the increased incidence of diabetes in this country has been termed an "epidemic". (Robert Steinbrook, MD, *The New England Journal of Medicine*, February 9, 2006; vol. 354: pp. 545-548, 545). One of the primary culprits for this increase in diabetes is poor glycemic control over the long term. (*Id.*) "One dietary strategy aimed at improving both diabetes control and cardiovascular risk factors is the use of low–glycemic index diets. These diets have been reported to benefit the control of diabetes ... and reduce diabetes incidence and overall cardiovascular events." (David J. A. Jenkins, MD et al., *The Journal of the American Medical Association*, Dec. 17, 2008; vol. 300: pp. 2742-2753, 2742).

Foods with a high glycemic index rapidly release glucose into the bloodstream and stress the body's insulin system. In contrast, foods with a lower glycemic index have a slower digestion period, more gradual glucose release, and provide a sustained energy release thereby providing better long term blood glucose control. Many foods are sweetened with conventional high-glycemic sweeteners such as corn syrup. Moreover, corn syrup often makes up a significant portion of the caloric content of these foods. It would be beneficial if a sweetener could be used in place of corn syrup in foodstuffs which has a similar functionality to corn syrup, i.e. similar caloric content and similar use in foodstuffs, but has a lower glycemic index than corn syrup.

The present invention relates to such a low-glycemic sweetener. This low-glycemic sweetener has a "level of sweetness similar to that of a corn syrup, and a mouth-feel and functionality similar to that of corn syrup." (Specification, p. 2; emphasis added). Thus, the present invention acts as a substitute for corn syrup in foodstuffs, with the advantage of having a lower glycemic index.

The present invention differs from Examiner's cited prior art references in 3 important ways. First, the present invention is a low-glycemic sweetener. The carbohydrates taught by the prior art references do not release glucose into the bloodstream and, as explained above, may not be categorized as "low-glycemic". Second, the present invention is a full calorie sweetener. The

carbohydrates taught by Examiner's cited prior art references are zero calorie additives used as bulking agents, fillers, and extenders in foodstuffs. The present invention is used in place of corn syrup in foodstuffs and replaces the calories which corn syrup would normally provide to a foodstuff. Third, the present invention may be used as a sweetener on its own. This is in stark contrast to the carbohydrates taught in both of the Examiner's cited prior art references. The carbohydrates taught in these references must be combined with a high intensity sweetener and cannot be used as sweeteners on their own. These carbohydrates simply function as zero calorie bulking agents or extenders for high intensity sweeteners such as aspartame.

The present invention is a low-glycemic sweetener. As described above, glycemic index is correlatable with glucose release. A carbohydrate which has a glycemic index is broken down during digestion and releases glucose into the bloodstream. The carbohydrates taught in the Examiner's cited prior art references are zero calorie food additives used as fillers, extenders or bulking agents. These carbohydrates are, by definition, not broken down during digestion and therefore do not release glucose into the bloodstream. Because these carbohydrates do not release glucose into the bloodstream, they do not have a glycemic index.

The present invention is also a full calorie sweetener. Unlike a typical sweetener such as sucrose or corn syrup, the sweetener of the present invention has a low glycemic index, meaning it will gradually release glucose into the bloodstream during the digestive process. However, a low glycemic index does not equate to a low caloric content, and likewise, a low caloric content does not equate to a low glycemic index. On the contrary, while the present invention is a low-glycemic sweetener, one of its important functionalities is to replace the caloric content in foodstuffs which would have been provided by a conventional sweetener such as corn syrup. This functionality as a calorie or energy replacer can be seen in Example 7 of the specification. Example 7 recites a list of food compositions that may be prepared utilizing the low-glycemic sweetener of the present invention instead of corn syrups. (See Specification, pp. 17-20). Of particular note is the use of the low-glycemic sweetener in meal replacement bars and meal replacement beverages. The purpose of a meal replacement bar or a meal replacement beverage

is to replace calories which would normally have been ingested through the course of a meal. The zero calorie carbohydrates taught by the Examiner's cited prior art references could never be used in this capacity - for meal replacement - because they would not replace the calories lost due to a skipped meal. However, because the present invention is a full calorie sweetener, it is an appropriate substitute for conventional sweeteners like corn syrup used in meal replacement bars and meal replacement beverages.

The present invention also acts as a sweetener on its own. Specifically, it does not need to be combined with other compounds to act as a sweetener. This is in stark contrast to both of Examiner's cited prior art references. The carbohydrates taught in both of these references are used as bulking agents, extenders, or fillers, but are not sweeteners on their own. Rather, they are used in conjunction with other compounds to function as sweeteners. These 3 significant differences distinguish the present invention from Paul and Leathers.

Paul, in contrast to the present invention, does not teach a carbohydrate which can be characterized either as "low-glycemic" or as full calorie, and furthermore does not teach a carbohydrate which can be used as a sweetener on its own. The carbohydrate in Paul contains at least one $\alpha(1\rightarrow2)$ glucoside bond (Paul, col. 1, l. 20). Oligosaccharides with these linkages are generally non-digestible. According to Paul, this bond is particularly resistant to enzymatic hydrolysis by glucohydrolase enzymes. (Paul, col. 2, ll. 9-10). These are enzymes which cleave glucose molecules from the oligosaccharide. The carbohydrate taught in Paul will not release glucose into the bloodstream during digestion. It therefore simply cannot be characterized as having a glycemic index of any kind, and consequently cannot be "low-glycemic".

In addition, the carbohydrate of Paul is not a full calorie sweetener. Instead, it is "useful as fillers or extenders in sugar substitutes which are metabolizable by man only slightly or not at all." (Paul, col. 2, ll. 16-18). This is in stark contrast to the full calorie sweetener of the present invention, which is specifically used in calorie replacement. Unlike the present invention, the oligodextran of Paul would not be used in place of the corn syrups in a meal replacement bar or

meal replacement beverage. Indeed, the function of these foodstuffs would be greatly undermined if the oligodextran taught in Paul were used, since these foodstuffs would no longer adequately replace calories and provide the energy release normally associated with meal replacement bars and meal replacement beverages. Finally, the carbohydrate taught in Paul is not a sweetener on its own. Rather, it is utilized in conjunction with an artificial sweetener. Specifically, it may be "used in low calorie foodstuff formulations, mixed with a strong sweetener, such as aspartame or equivalent." (Paul, col. 2, ll. 19-22).

Like Paul, the carbohydrates taught in Leathers, alternan and alternan derivatives, are also notably different from the low-glycemic sweetener of the present invention. These carbohydrates are not low-glycemic, are not full-calorie, and cannot be used as sweeteners on their own. Leathers states that "alternan and alternan derivatives have potential value as noncaloric, carbohydrate-based soluble food additives in artificially sweetened foods." (Paul, col. 1, ll. 33-35; emphasis added). The fact that these carbohydrates are "noncaloric" indicates that they will not be digested to release glucose into the bloodstream. In addition, in light of the fact that these carbohydrates are "noncaloric", it is readily apparent that they could never be considered full calorie. Finally, the carbohydrates taught in Leathers also are not used as sweeteners on their own. Rather, as stated in Leathers, "[a]lternan has potential commercial applications as a low-viscosity bulking agent and extender in foods ...." The carbohydrates taught in Leathers are used as bulking agents, extenders and food additives; they are not used as sweeteners on their own.

The 3 major differences described above serve to distinguish the present invention from Paul and Leathers. In the Rejection, the Examiner stated that "[Paul] teaches that the oligodextrans produced by the invention are particularly resistant to enzymatic hydrolysis by glucohydrolase enzymes. This property makes them useful as fillers or extenders in sugar substitutes which are metabolizable by man only slightly or not at all (i.e. low glycemic index material). They may therefore be used in low calorie foodstuff formulations." (Rejection, p. 3; emphasis added). Examiner mischaracterizes the meaning of "low-glycemic". As described above, glycemic index is correlatable to glucose release. Glycemic index is not related to caloric

intake. Neither Paul nor Leathers mentions glycemic index nor do they state that the carbohydrates they teach are low-glycemic. Indeed, they could not, since the carbohydrates taught by these references do not release glucose into the bloodstream and therefore do not have a glycemic index.

Moreover, the fact that the carbohydrates taught in Paul may be used in low calorie foodstuff formulations does not indicate that they are digestible. Indeed, it is precisely the fact that these carbohydrates are not digestible which makes them effective in low calorie foodstuff formulations – they add bulk to foodstuffs without adding calories.

The Examiner concluded the Rejection by stating that "based on the combined teachings of [Paul and Leathers], there would be a reasonable expectation of success in making low glycemic index sweeteners." (Rejection, p. 4) Applicants contend that a person of skill in the art would have no reasonable expectation that, based on Paul and Leathers, it would be possible to make the low-glycemic full calorie sweetener of the present invention. Moreover, a person of ordinary skill in the art would not be motivated to modify or combine these references to obtain the present invention. Neither reference teaches a carbohydrate which can be characterized as "low-glycemic", or full calorie, or can act as a sweetener on its own. In fact, a low-glycemic full calorie carbohydrate which can be used as a sweetener on its own would not be contemplated by a person of skill in the art having read Paul and Leathers. In light of the disclosures in these references, the present invention is a surprising technical achievement.

Applicants additionally submit that a person normally skilled in the art, searching for a method to make a low-glycemic full calorie carbohydrate which can be used as a sweetener on its own, would not examine the references cited by the Examiner alone or in combination. Neither reference teaches a carbohydrate with <u>any</u> of the desired characteristics. In fact, these references are inapposite to the present invention. Paul and Leathers teach non-digestible carbohydrates used as fillers, extenders or bulking agents. The low-glycemic sweetener of the present invention is specifically intended to be ingested, and provide caloric intake similar to conventional high-

glycemic sweeteners such as corn syrup. Indeed, the present invention would not be identified as possible by a person of ordinary skill having read Paul and Leathers.

For these reasons Applicants respectfully request that the rejection under 35 U.S.C. § 103 be withdrawn, and the pending claims be allowed.

Please apply any charges or credits to deposit account 50-2342.

Respectfully submitted,

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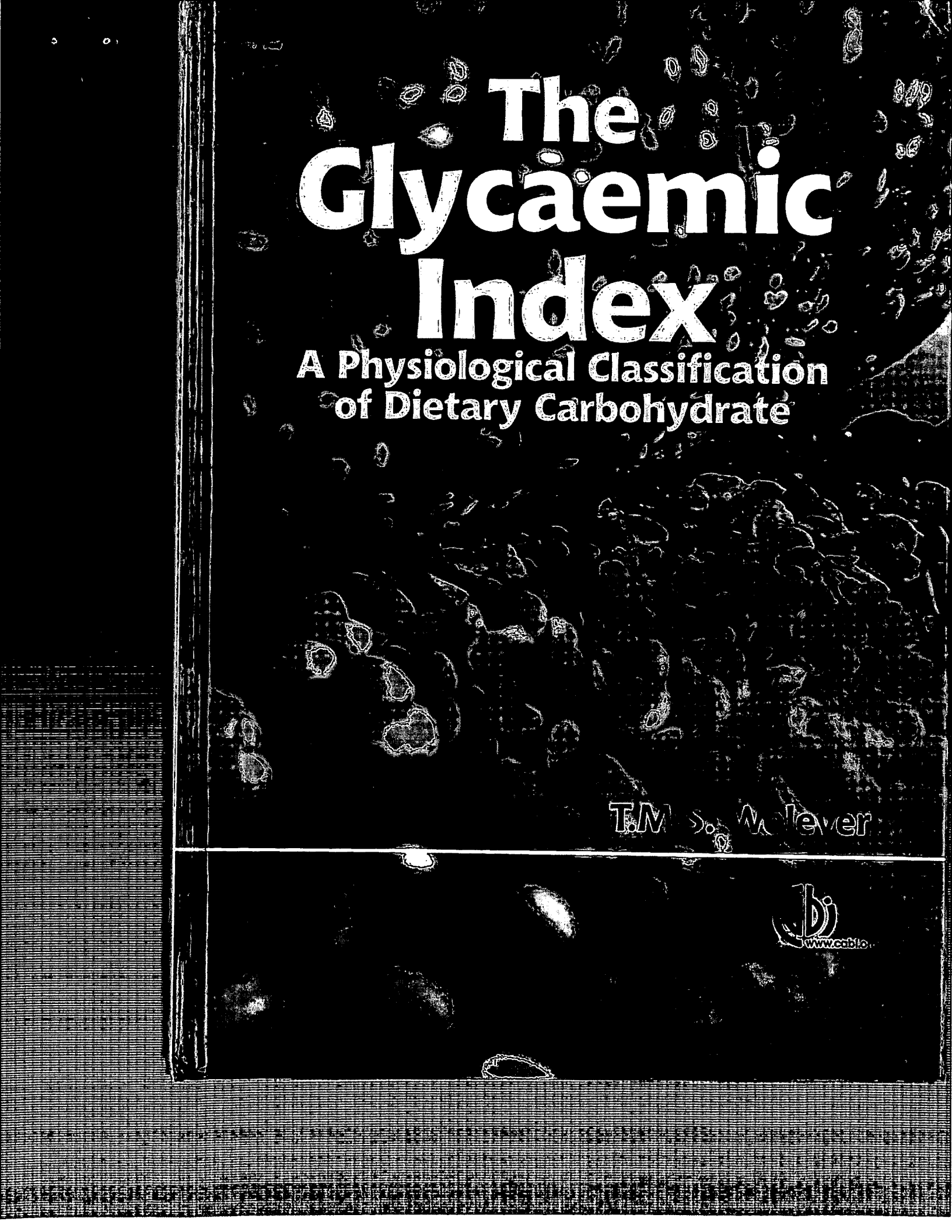
The Glycaemic Index
A Physiological Classification of Dietary Carbohydrate
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2

Determining the GI of Foods – Methodological Considerations

To be practically useful, the definition of the term 'glycaemic index' and the methods used to determine the GI of foods must be standardized so that the values obtained are not only reproducible but also mean the same thing in different countries. Unfortunately, the definition of certain nutrients differs in different countries. Most unfortunately for the GI, dietary carbohydrates fall into this category, with long-standing disagreements about the terminology used to describe the term 'carbohydrate' and its components from a regulatory point of view. In North America, the amount of 'carbohydrate' on the food label includes total carbohydrate measured by difference, whereas in Europe, 'carbohydrate' on the food label includes available carbohydrates measured directly. Similar problems occur with the term 'dietary fibre' and 'starch'. I believe these disagreements have contributed to misunderstanding and confusion about the GI, and are certainly the source of differences in methodology used to determine the GI of foods.

The method used to determine the GI of foods is deceptively complex with numerous issues to be considered. The importance of some issues, such as how AUC is calculated, or how 'carbohydrate' is defined, is sometimes not appreciated, and the method used not described in some papers on GI. In these cases, it is difficult to compare the results with other data in the literature. On the other hand, some investigators go to great lengths to control and describe factors, such as careful selection of subjects without personal or family history of diabetes, which actually have little or no impact on the GI values obtained. In addition, there are a number of important issues about methods which have not been resolved, and there are many questions for which no data exist. What follows in this chapter is a description of the recommended method for determining the GI of foods, and a detailed justification as to why things are done that way, and, where data exist, the consequences of using different methods.

2.1 Definition of the 'Glycaemic Index'

The GI is defined as the incremental area under the blood glucose response curve elicited by a 50 g available carbohydrate portion of a food expressed as a percentage of the response after 50 g anhydrous glucose taken by the same subject.

This definition raises many questions such as: What is 'incremental area under the curve?' How is 'blood glucose' measured? What is 'available carbohydrate?' What kind of subjects should be studied? Does it have to be a 50 g carbohydrate portion? Does the reference food have to be glucose? Before addressing these issues, it is important to consider questions of a more philosophical nature, the answers to which must take into account the purpose of the GI, and what distinguishes GI from information about food composition which can be obtained from chemical analysis. These issues relate to the 'meaning' of the GI rather than its definition.

2.1.1 Meaning of 'glycaemic index'

The GI was originally meant to be an index of the blood glucose raising potential of the available

carbohydrate in foods. Monro (2002) has shown that GI does not indicate the glycaemic impact of a food. He rightly points out that GI is a property of the carbohydrates in foods, not a property of foods, and also that GI is a value which is independent of the portion size of the food or the amount of carbohydrate consumed. He believes, therefore, that GI is '...not suitable for dietary management of postprandial glycaemia....', and should be redefined (Monro, 2003). However, I believe current evidence suggests that the original meaning of GI should be retained because it provides a unique and useful measure of the biological quality of the available carbohydrate in foods. I will review this evidence in Chapter 9. However, here it is important to point out a number of principles that follow from this meaning.

2.1.1.1 *Glycaemic index is not the same as glycaemic response*

The term 'glycaemic index' is often used incorrectly to mean 'glycaemic response' in a variety of situations including: mixed meals, foods containing unavailable carbohydrate and differences between subjects. In the context of mixed meals, for example, it has been stated that: 'Adding fat to bread reduces its glycaemic index'. In this case, the correct terminology would be: 'Adding fat to bread reduces the glycaemic response'. Also, investigators sometimes determine the glycaemic responses of mixed meals and calculate a 'GI' of the meals by expressing the glycaemic responses as a percentage of the response of the same subjects to bread or glucose. This is inappropriate because the GI is a property of individual carbohydrate foods tested by themselves with nothing added. It is known that fat and protein affect glycaemic responses, but these effects have nothing to do with the glycaemic response of the carbohydrate. In addition, the effects of added fat and protein on glycaemic responses differ in normal subjects, subjects with type 1 diabetes and subjects with type 2 diabetes (discussed in Chapter 5, this volume). On the other hand, the GI of individual carbohydrate foods is the same in all of these different types of subjects. Thus, the GI of mixed meals should be calculated from the GI values of the individual foods whereas the 'glycaemic responses' of mixed meals should be measured *in vivo*. The relative differences between the observed glycaemic responses should be predicted by the relative differences in calculated meal GI.

Some investigators persist in using the term 'glycaemic index' to describe the fact that foods containing low amounts of available carbohydrate, such as vegetables, have low glycaemic responses (Tremblay *et al.*, 2002). This is inappropriate because it is possible to tell from the food label that a food has little or no carbohydrate, and thus will not raise blood glucose. We can also know from the food label how much unavailable carbohydrate (non-starch polysaccharides (NSP), inulin, etc.) a food contains and since, by definition, unavailable carbohydrate is not available to the body as glucose, it does not raise blood glucose. A food in which some of the available carbohydrate has been replaced by unavailable carbohydrate (e.g. resistant starch (RS)) will elicit a lower glycaemic response than the normal food fed at the same level of total carbohydrate, but this could be known from the label, because the amount of available carbohydrate is less. It is not correct to equate this with a low-GI food because the long-term effects of reducing available carbohydrate intake are not the same as reducing the GI (Chapter 9, this volume).

The terms 'glycaemic index' and 'glycaemic response' should also not be confused because these entities have different mathematical and statistical properties (Wolever, 1992). Theoretically, the GI adjusts glycaemic response areas to each individual's response to a reference food, thus correcting for between-subject variation. In order to test this hypothesis, we determined the glycaemic responses of bread, rice and spaghetti in 12 subjects with diabetes with each subject repeating each food four times (Wolever *et al.*, 1990). The results showed that the glycaemic responses (i.e. incremental AUC values) differed significantly for the different foods and also differed significantly in the different subjects. Indeed, 62% of the total variance was accounted for by variation between subjects (Fig. 2.1). The exact magnitude of between-subject variation of glycaemic responses depends on the homogeneity of the subjects chosen, and in this case was large because subjects were chosen to be dissimilar by including subjects with both type 1 and type 2 diabetes on a variety of different treatments. However, when the AUC values were expressed as a percentage of each subject's average AUC after white bread (i.e. the GI), the difference between subjects was no

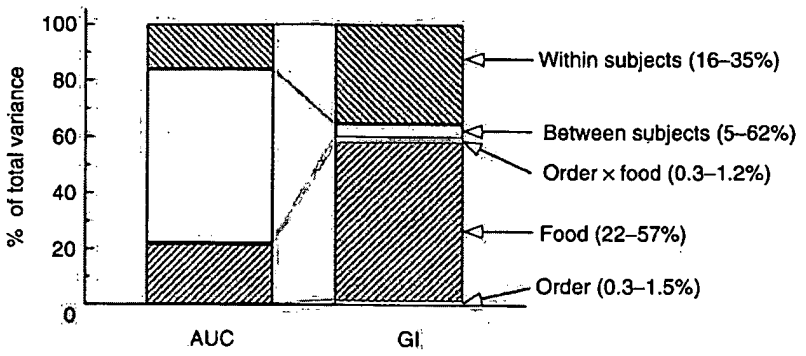

Fig. 2.1. Partitioning of variance of incremental areas under the glycaemic response curve (AUC) and glycaemic index values (GI) from tests in 12 subjects with diabetes whose glycaemic responses were measured after consuming bread, rice and spaghetti with each subject repeating each food four times (Wolever *et al.*, 1990).

longer significant, with between-subject variation accounting for only 5% of the total variance (Fig. 2.1). The proportion of total variance explained by variation within-subjects and variation between foods increased by more than twofold when results were expressed as GI. However, this is because the reduction in between-subject variation reduces total variation; when expressed as coefficient of variation (CV = 100 × SD/mean), expressing results as GI reduces between-subject variation without changing variation between foods or variation within subjects (Table 2.1).

2.1.1.2 *Low-carbohydrate foods*

Foods containing no carbohydrate, such as bacon or eggs, do not have a GI. The GI is not an index of how much the food raises blood glucose, it is an index of the extent to which the available carbohydrate in the food raises the blood glucose.

It was originally intended that the GI should apply to 'high' carbohydrate foods such as grains, breads, cereals, etc. A problem arises when one considers manufactured food products which contain a considerable amount of their energy as fat and protein, such as meal replacement bars, or certain natural foods such as dairy products and nuts. It is known that fat and protein may affect glycaemic responses, as will be discussed in Chapter 5. Thus, the glycaemic response elicited by foods containing large amounts of fat and protein may be low because of the effects of fat and protein on insulin secretion or gastric emptying, rather than because of the intrinsic nature of the carbohydrate.

For example, let us consider a food bar such as Ironman PR Bar (PR Nutrition, San Diego, California) containing about 40% energy as carbohydrate, 30% fat and 30% protein, the main carbohydrate ingredients of which are high-fructose corn syrup (HFCS) and sucrose (University of Arizona, accessed 12 January, 2005). A 50 g available carbohydrate portion of this bar contains about 17 g fat and 35 g protein,

Table 2.1. Sources of variation of incremental area under the glycaemic response curve (AUC) and glycaemic index values (GI) expressed as standard deviations (SD) and coefficient of variation (CV).[a]

	AUC values		GI values	
Source of variance	SD	CV (%)	SD	CV (%)
Food	262	31	22.9	30
Within subjects	210	25	15.9	21
Between subjects	408	49	5.8	8

[a]Data from Wolever *et al.* (1990) and Wolever (1992).

and elicits a mean glycaemic response about 55% that of a 50 g carbohydrate portion of white bread (Hertzler, 2000). Thus, the approximate GI of the bar is 55 × 0.71 = 39, a value 35% less than the GI of its carbohydrate ingredients (taken to be sucrose, GI = 60). However, it must be noted that my estimate of the GI of the carbohydrate ingredients assumes the HFCS in the Ironman PR bar is a 50:50 mixture of glucose and fructose; if the HFCS contained predominantly fructose, then the expected GI would be lower, but if the HFCS was predominantly glucose, then the expected GI would be higher (Chapter 5, this volume describes how to calculate the GI of a mixture of carbohydrate foods).

Tests on other types of food bars and candy bars suggest that, in the context of a food bar, protein may have more impact on the glycaemic response than fat. Hertzler (2000) showed that a 50 g available carbohydrate portion of PowerBar (Powerfood, Berkeley, California), containing 2 g fat and 12 g protein, elicited a mean glycaemic response 75% that of bread. PowerBar contains 40–45% of carbohydrate as glucose and fructose (mean GI = 60) and 50–55% as maltodextrin (GI = 100), brown rice (GI = 90) and oat bran (GI = 56) (PowerBar Sport When To Use/FAQ), thus (assuming oat bran to be a relatively minor ingredient) the GI of its carbohydrate ingredients is likely between 70 and 80, compared to the observed approximate GI of 75 × 0.71 = 53. On the other hand, 50 g available carbohydrate portions of Snickers candy bar (M&M Mars, Hackettstown, New Jersey) and Mars candy bar contain only 2–4 g protein but 14–21 g fat. The major carbohydrates in these candy bars are sugar (GI = 60) and corn syrup (GI = 100) so, assuming somewhat more sugar than corn syrup, the GI value of the carbohydrate mixture is likely between 70 and 80. Snickers elicited a mean glycaemic response 97% that of bread (GI ≈ 69) (Hertzler, 2000), and we found Mars bar to have a GI of 68 (Jenkins *et al.*, 1981a).

Thus, although the presence of some protein and fat do not necessarily have a major effect on the glycaemic response to carbohydrate, it is likely that high amounts do. This is an important issue, because if, as I believe it should be, the GI is a measure of the biological effect of the available carbohydrate in foods on glycaemic responses, independent of the effects of fat and protein on gastric emptying and insulin secretion, then it is not appropriate to determine GI on foods high in protein and fat. However, systematic dose–response studies have not been done on the effect on glycaemic responses of adding protein and fat alone and in combination to carbohydrate. Thus, it is difficult to determine a threshold for protein and fat contents of moderate and low-carbohydrate foods above which it might be considered inappropriate to determine GI.

2.1.1.3 GI is measured for single foods and calculated for mixed meals

The GI has been criticized because it is a property of single foods which may not reflect the glycaemic effects of foods when they are consumed in a mixed meal due to the effects of added fat and protein (Hollenbeck *et al.*, 1986; American Diabetes Association, 1994, 2002; Franz *et al.*, 1994, 2002). To this end, some investigators are now measuring GI values of mixed meals by expressing the glycaemic response of a mixed meal containing 50 g carbohydrate as a percentage of the response after 50 g glucose or 50 g carbohydrate from bread. This approach is problematic because the magnitude of the difference in glycaemic response between the reference food and the mixed meal is not only due to differences in the carbohydrate, but also to the added fat and protein. The RGR of foods are the same in different groups regardless of their glucose tolerance status (see Section 2.2.4.1). However, this is not so for fat and protein. The effects of fat and protein on glycaemic responses have not been systematically studied, but it is known that the effects are influenced by different sources of fat and protein, the habitual diet the subject is consuming, the way the fat and protein is incorporated into the meal, and most importantly, the glucose tolerance status of the subjects (these issues are reviewed in Chapter 4, this volume). The implication of this is that the glycaemic effect of a mixed meal containing fat and protein, relative to that elicited by a reference food without fat and protein, may not be the same in different subjects. Therefore, the results of studies where the GI of mixed meals is measured are likely to be only applicable to the specific meal and in the specific group of subjects studied.

However, the GI values measured in individual foods can be applied to gain an insight into the relative glycaemic effects of mixed meals containing fat and protein in both normal and diabetic

subjects. To do this, the GI of the mixed meal is mathematically *calculated* from the GI values and carbohydrate contents of the individual foods present in the mixed meal. This calculation ignores the effects of added fat and protein. The way this is done, and the validity of the approach is reviewed in Chapter 4. If done using the appropriate methods, the calculated GI of mixed meals is usually very closely related to the observed glycaemic responses elicited by the meals. Even if this were not so, the approach of determining the glycaemic effects of foods individually before combining them into a mixed meal is useful for helping to understand what determines the glycaemic responses of mixed meals.

The point being made here is that the GI is *measured* in individual foods and *calculated* for mixed meals. Thus, the criticism that the GI is not useful because it is only measured in single foods is way off the mark. Far from being a weakness, the fact that the GI is measured only in single foods is a major strength of the concept and is precisely what allows the GI to be useful.

2.1.1.4 Exclusion of non-glycaemic carbohydrates

The GI is intended to be a measure of how much the available carbohydrate in a high-carbohydrate food raises blood glucose. Therefore, unavailable, or non-glycaemic, carbohydrates should be excluded from the 50 g carbohydrate portion because, by definition, these carbohydrates do not raise blood glucose. When the GI was first developed, this was a fairly simple concept to apply in practice, because the only 'unavailable' carbohydrate for which analytic data were available was 'dietary fibre'. However, unavailable or partially available carbohydrates now include RS, unavailable oligosaccharides (e.g. inulin and fructo-oligosaccharides), modified starches, polydextrose and sugar alcohols, and these compounds are appearing more and more in the food supply. It is now appreciated that it is difficult, if not impossible, to measure the amount of carbohydrate in foods which is actually absorbed from the human small intestine. A method for measuring RS, which is accepted by the Association of Official Analytical Chemists, has been developed (McCleary and Monaghan, 2002). However, measured RS may overestimate the amount of carbohydrate entering the colon after consumption of starchy foods (Englyst and Cummings, 1986, 1987; Danjo *et al.*, 2003), particularly for amounts of RS over 1 g (Fig. 2.2). Most polyols are partly absorbed, and it is not only difficult to determine exactly how much is absorbed, but it is known that the proportion of the amount consumed which is absorbed probably varies in different individuals and also varies depending on whether they are consumed alone or with other foods (Beaugerie *et al.*, 1990). In addition, a proportion of some sugar alcohols which are absorbed are not metabolized, but excreted intact in the urine. Livesey (2003) reviewed the metabolism of polyols recently, and provided a useful table showing the approximate absorption and urinary excretion of different kinds of sugar alcohols which can be used to estimate available carbohydrate for GI determinations (Table 2.2).

Since it is difficult or impossible to actually measure the amount of 'available' carbohydrate in foods, it could be argued that most of the GI measurements in the literature have not properly excluded unavailable carbohydrate, and that the reductions in GI could be due to reductions in carbohydrate absorption. From this, it could be argued that unavailable carbohydrate should not be excluded from the 50 g carbohydrate load.

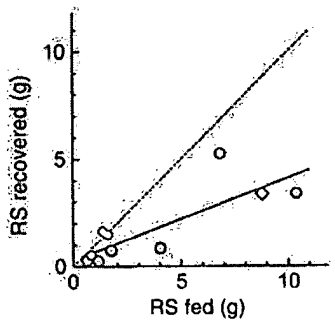

Fig. 2.2. Amount of resistant starch (RS) fed vs the amount recovered from the terminal ileum. (○) Mean recovery of RS in potatoes from groups of two to five subjects with an ileostomy (Englyst and Cummings, 1987); (○) recovery of RS in banana from individual subjects with an ileostomy (Englyst and Cummings, 1986); (◇) mean recovery of RS in high amylose maize starch from seven normal volunteers measured by intubation (Danjo *et al.*, 2003). Dotted line = line of identity; solid line = regression line.

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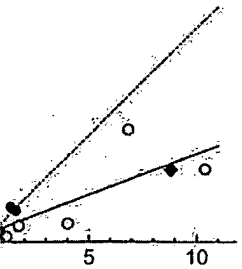

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Table 2.2. Approximate absorption and excretion of polyols.

	Absorption (%)	Urinary excretion (%)	Available[a] (%)
Erythritol	90	90	0
Xylitol	50	<2	50
Sorbitol	25	<2	25
Mannitol	25	25	25
Isomalt	10	<2	10
Lactitol	2	<2	0
Maltitol	40	<2	40
Maltitol syrup	45	<2	45
Polyglycitol	40	<2	40

[a]Per cent (g/100 g) to be included as 'available carbohydrate' for GI determinations.
Adapted from Livesey (2003).

However, the latter would only follow if the former were true. I will show in the next chapter that there are differences in the amount of carbohydrate absorbed from different starchy foods, but that these are not nearly large enough to explain the differences observed in their GI values. Errors, which may exist in the literature due to not accounting for unavailable carbohydrate, are so small as to be virtually non-detectable without a huge increase in the number of subjects used to determine GI values, even for foods naturally containing large amounts of RS. Therefore, to retain the meaning of the GI, unavailable carbohydrates should be excluded.

2.1.2 Suggested protocol for determining the GI of foods

2.1.2.1 Subjects

Male or non-pregnant and non-lactating female subjects aged 18–75 years can be included. For most purposes, healthy subjects with normal glucose tolerance are used, but subjects with IGT or diabetes may be included. The inclusion of pregnant or lactating females or subjects with unstable diabetes mellitus is not recommended because the carbohydrate tolerance status of such subjects is changing rapidly with time, and therefore the subject may not be in the same state when the glycaemic responses of the test and reference foods are determined. The number of subjects included depends on the magnitude of the confidence interval (CI) desired. For most purposes, use of ten subjects is recommended.

2.1.2.2 Procedures

Subjects are studied in the morning (between 7:00 and 9:30 am) after an overnight fast of 10–14 h. On each test occasion the subject is weighed, and a fasting blood sample is obtained by finger-prick. Then the subject starts to consume a test meal. When the subject takes the first bite of the test meal, a timer is started and additional blood samples are taken by finger-prick at 15, 30, 45, 60, 90 and 120 min after starting to eat. After the last blood sample, the subject is offered tea or coffee and a snack and is free to leave. During the 2 h of the test, subjects remain seated quietly. Whole blood or plasma glucose is measured using any recognized reference method.

The test meals consist of portions of the test food containing 50 g available carbohydrate. If this results in a portion size, which is too large to consume by the subjects, the amount of available carbohydrate can be reduced. GI values based on 25 g available carbohydrate portion sizes have been published. The effect of reducing the amount of carbohydrate in the portion will be discussed below. Ideally available carbohydrate is based on direct measurement and does not include RS or other unavailable carbohydrates. If the food contains carbohydrates which are partly absorbed and/or not metabolized (e.g. sugar alcohols), the unabsorbed and/or non-metabolized portion should not be included in the 50 g available carbohydrate load (Table 2.2). In North America, methods for determining carbohydrate differ, and it is acceptable to define available carbohydrate as total carbohydrate minus dietary fibre measured by the AOAC method, minus any unavailable carbohydrate ingredients not included in dietary fibre. In the absence of definitive data, the proportions of carbohydrates absorbed and metabolized may have to be estimated from the available literature.

Subjects should have a drink with the test meal, and can choose to have one or two cups of water, tea or coffee with or without 2% milk and artificial sweetener; the volume and type of drink the subject chooses should remain the same for all tests done by that subject. Test meals are consumed within 10 min.

Subjects are studied for a series of tests including a certain number of test foods and at least three tests of the reference food (anhydrous glucose or white bread are commonly used). Generally, the reference food tests are done at the beginning, middle and end of a series with the order of the other foods randomized. If more than 12 foods are tested at one time, one reference standard generally should be included for every 3 months, or for every five to six test foods (whichever is sooner) to ensure that the subjects' carbohydrate tolerance is not changing with time.

The incremental area under the curve (IAUC) under each blood glucose response curve is calculated as follows:

For times $t_0, t_1, \ldots, t_n$, the blood glucose concentrations are $G_0, G_1, \ldots, G_n$, respectively. IAUC $= \sum_{n}^{x=1} A_x$ where A_x = the IAUC for the xth time interval (i.e. between t_{x-1} and t_x).

For the first time interval (i.e. $x = 1$): if $G_1 > G_0$, $A_1 = (G_1 - G_0) \times (t_1 - t_0)/2$
otherwise, $A_1 = 0$

For the other time intervals (i.e. $x > 1$)
if $G_x \geq G_0$ and $G_{x-1} \geq G_0$, $A_x = \{[(G_x - G_0)/2] + [(G_{x-1} - G_0)/2]\} \times (t_x - t_{x-1})$
if $G_x > G_0$ and $G_{x-1} < G_0$, $A_x = [(G_x - G_0)^2/(G_x - G_{x-1})] \times (t_x - t_{x-1})/2$
if $G_x < G_0$ and $G_{x-1} > G_0$, $A_x = [(G_{x-1} - G_0)^2/(G_{x-1} - G_x)] \times (t_x - t_{x-1})/2$
if $G_x \leq G_0$ and $G_{x-1} \leq G_0$, $A_x = 0$

The individual IAUC values for each test food in each subject are expressed as a percentage of the mean IAUC value for the repeated reference food tests taken by the same subject. The mean of the resulting values for each food is the GI value for that food.

2.1.3 Performance of method

An interlaboratory study was conducted recently in which seven centres experienced in GI methodology participated (Toronto, Canada; Sydney, Australia; Dunedin, New Zealand; Potchefstroom, South Africa; Milan, Italy; Lund, Sweden; and Trinidad and Tobago). Each laboratory determined the GI of four centrally provided foods and locally obtained white bread, using centrally provided anhydrous glucose. Two centres collected venous blood and five collected capillary blood by finger-prick. The variation of the results was greater for the two centres which collected venous plasma, which probably reflects a real difference between venous and capillary blood sampling, as will be discussed below. The average SD of the centre mean GI values for the five foods was 9 (Wolever *et al.*, 2003a).

2.2 Effects of Variation in Methods

2.2.1 Calculation of AUC

There are many ways of calculating the AUC. Major differences in results or interpretation of the results may be obtained from the same data depending on how the AUC is calculated (Wolever and Jenkins, 1986; Ha *et al.*, 1992). One consideration is whether the trapezoid rule can be used to calculate the AUC, or whether a more sophisticated and complex model should be used. The other consideration is the area which is included in the AUC.

The AUC is estimated based on measures of blood glucose concentration obtained at various instants in time. Ideally, AUC should be based on blood glucose concentrations measured continuously. This is at present impossible to achieve and even indwelling devices to measure blood glucose 'continuously' actually produce measurements at intervals of approximately 30–60 s. Usually, glucose is measured at 15–30 min intervals, and to determine AUC one has to estimate what is happening to blood glucose between the times when it is measured. The simplest method is to draw a straight line between each point, and this is the basis of the trapezoid rule. This is not physiological. A more physiological approach would be to connect the points when glucose is measured with a smooth curve (Fig. 2.3). The problem with this is that it is difficult to know what should be the shape of this curve. In addition, the calculations involved are complex. When the trapezoid method was compared with polynomial interpolation of third and fourth degree, Simpson's integration and cubic interpolatory splines, the different methods produced slightly different results, but the variation was no more than 2%, and the estimates were highly correlated with each

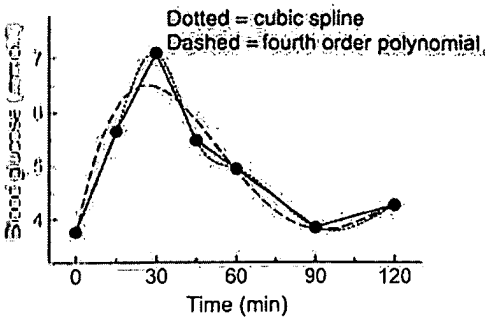

Fig. 2.3. Examples of models to generate a smooth glycaemic response curve from data points (•) compared with straight lines connecting the points.

other ($r > 0.998$) (Le Floch *et al.*, 1990). Thus, it appears that the trapezoid method is suitable for calculating AUC. The next question is what area should be included.

Different ways of calculating the AUC are shown in Fig. 2.4. The GI is based on the IAUC, defined as the area beneath the curve above the fasting level only; area beneath the fasting level is ignored. Thus, IAUC cannot have a value < 0. It was initially used because it indicates the amount to which the food raises blood glucose above the fasting concentration. Net incremental AUC (netAUC) (Gannon *et al.*, 1989) includes all area below the curve, including area below the fasting concentration. Since it is calculated by applying the trapezoid rule to both positive and negative blood glucose increments, the effect is to subtract area below the fasting level from that above.

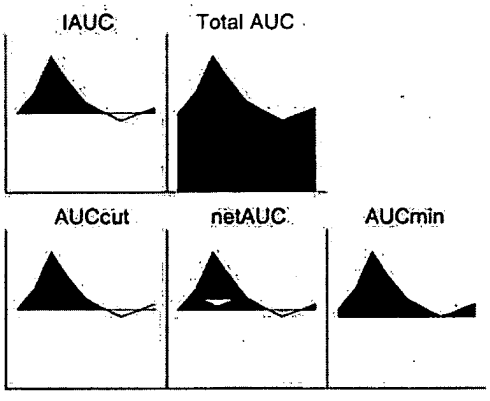

Fig. 2.4. Area subtended by different methods used to calculate area under the curve.

Thus, netAUC can have a value < 0. AUCcut (Ha *et al.*, 1992) is calculated in the same way as IAUC, but only includes area before the blood glucose concentration drops below (cuts) the baseline (fasting concentration); area after the glucose concentration cuts the baseline is not included. IAUC above the lowest blood glucose concentration attained (AUCmin) (Vorster *et al.*, 1990) is calculated by subtracting the lowest blood glucose concentration attained during the test period from each of the other blood glucose concentrations, and calculating the AUC by applying the trapezoid rule to the resulting increments. Total AUC (TAUC) is the area under the blood glucose response curve above a blood glucose concentration of zero.

TAUC of blood glucose always has a value much greater than that for the incremental methods, because much of the TAUC is determined by the concentration of fasting glucose. In subjects with diabetes, about 80% of the variance in TAUC is determined by the basal or fasting glucose concentration (Le Floch *et al.*, 1990). The different methods of calculating IAUC will all have the same value if the postprandial blood glucose concentrations obtained are all greater than the fasting level. The different methods produce different results if blood glucose drops below the fasting level. This is illustrated in Table 2.3 which shows the results of sample calculations on two sample blood glucose profiles using different methods of calculating AUC. Table 2.3 also shows that the different methods of calculating AUC can result in markedly different values when the AUC for the food is expressed as a percentage of that after glucose.

There is no 'right' or 'wrong' way of calculating AUC; indeed, different methods may be most appropriate depending on the purpose of the analysis. Unfortunately, in some of the literature, the method of calculating AUC is not indicated, and it can, therefore, be difficult to know if the magnitudes of the relative effects of different test meals found in one study are comparable to those found in another. For the GI to be a valid and useful property of foods, and for valid comparisons of experimental results with published GI values, a standardized method of calculating AUC must be used.

TAUC is useful for comparing the effects of different treatments on average blood glucose concentrations, but it is an insensitive method

Table 2.3. Example of different methods of calculating AUC.

	Blood glucose concentrations (mmol/l) at time (min)											
	0	15	30	45	60	90	120	IAUC	netAUC	AUCcut	AUCmin	TAUC
Glucose	3.67	6.11	6.06	4.44	3.17	3.61	4.00	85.9	75.9	81.7	135.9	516
Food	3.94	5.00	5.11	3.44	3.50	3.83	4.33	35.4	18.6	30.8	78.6	491
		Food AUC expressed as % of glucose AUC:						41	25	38	58	95

for detecting differences in response between foods, since most of the TAUC is determined by the fasting glucose concentration, which is not affected by the test meal about to be consumed. We have shown that using netAUC and AUCmin result in GI values which are more variable (less precise) than IAUC. AUCcut performs similarly to IAUC, but is more difficult to calculate than IAUC, and in a study involving five foods, resulted in the SD of GI value of each of five foods being slightly higher (by 0.2–1.2) than those based on IAUC. Thus, IAUC appears to be the best method to calculate GI values (Wolever, 2004a).

2.2.2 Amount of 'available carbohydrate' in the portion tested

2.2.2.1 Definition of 'available carbohydrate'

The term 'available carbohydrate' is somewhat ambiguous; it originally meant a carbohydrate that provided glucose for metabolism, but it could also mean a carbohydrate that provides energy to the body. Energy can be provided either by the monosaccharides absorbed in the small intestine, or from the SCFA produced during the fermentation of unabsorbed carbohydrates in the colon. For this reason, the FAO carbohydrate report (1998) recommended using the term 'glycaemic carbohydrate' to mean carbohydrates, which provide glucose for metabolism. According to this definition, glycaemic carbohydrates are those which are absorbed in the human small intestine and metabolized in the body rather than excreted in the urine. However, the term 'glycaemic carbohydrate' is not ideal, because glycaemic carbohydrates do not necessarily produce glucose during metabolism, for example, fructose, which is clearly an available or glycaemic carbohydrate, does not necessarily yield glucose, but can be oxidized directly for energy. Thus, in this book, I will continue to use the term 'available carbohydrate' to mean carbohydrates which are absorbed from the small intestine and metabolized in the body via pathways which can, at least potentially, yield glucose.

As has been indicated above, and will be discussed in more detail later, it is difficult, if not impossible, to determine precisely the physiologically available carbohydrate in foods. Therefore, we need to rely on methods which define available carbohydrate from a chemical point of view. Ideally, methods for analysing carbohydrates directly, including sugars, total and resistant starch and NSP (McCleary and Monaghan, 2002; Champ *et al.*, 2003) should be used to determine the available carbohydrate. Unfortunately, these are not used for regulatory purposes in many parts of the world, particularly the USA, in which carbohydrates are analysed by difference and dietary fibre by gravimetric methods, both of which can include non-carbohydrate components. However, the amount of RS contained in most natural foods, and not included in the measurement of dietary fibre is relatively small, and accounting for it by feeding a larger portion size is unlikely to have any significant impact on the glycaemic response obtained. For example, in a recent interlaboratory study (Wolever *et al.*, 2003a), one of the test foods was pearled barley, a food which contains more RS than most other foods. The portion size fed in the study, 79.6 g, was based on analysis of total carbohydrate by difference and total dietary fibre. One of the centres involved in the study, experienced in measuring RS, found that 15.2% of the starch in the barley was RS, and thus, the 79.6 g portion size actually contained 44.2 g glycaemic starch. Increasing carbohydrate intake from 44.2 to 50 g would be expected to increase the glycaemic response by about 8% (Wolever and Bolognesi, 1996a). When the portion size of barley was increased to 93.9 to adjust for its RS content,

etAUC	AUCcut	AUCmin	TAUC
75.9	81.7	135.9	516
18.6	30.8	78.6	491
25	38	58	95

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When the portion size of barley
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this resulted in a non-significant increase in the GI from 39.3 ± 13.1 to 44.4 ± 13.1 (mean ± SD, $n = 10$). Although the 13% higher GI value found for the higher portion size of barley was similar to the expected difference, over 300 subjects would be required for an 80% chance of detecting a difference of 8% at $P < 0.05$.

However, large amounts of RS or other unavailable carbohydrates can be added to foods in the manufacturing process. Since these are added, they can be accurately measured, and accounting for them in determining the portion size to feed is critical to the interpretation of the results. For example, foods enriched with RS elicit lower glycaemic responses than portions of control products containing an equal amount of total carbohydrate (Hoebler *et al.*, 1999). However, Jenkins *et al.* (1998) found that 50 g available carbohydrate (not including RS) portions of muffins or cereal containing about 33% and 45% of total starch as RS elicited the same glycaemic response as 50 g available carbohydrate portions of control muffins and cereals. This suggests that at least some types of RS added to foods may not slow the rate of absorption of the available starch and reduce blood glucose and insulin responses simply by displacing available carbohydrate. The long-term effects of this on blood glucose, insulin and lipids may not be the same as that of slowing carbohydrate absorption. The issue of reduced carbohydrate intake vs reduced GI is addressed in Chapter 9.

Determining the GI of foods containing polyols, and other partly absorbed carbohydrates, presents a special problem because it is difficult to measure their absorption and metabolism, few data exist, and the different polyols vary in the degree to which they are absorbed and metabolized. Nevertheless, enough data exist to estimate the proportion of carbohydrate which is absorbed and metabolized (Table 2.2), and, in the absence of definitive information, such estimates are, in my view, better than not making any correction. Indeed, errors as high as 20% have only a small effect on the GI value obtained, especially for carbohydrates which do not raise blood glucose appreciably.

2.2.2.2 *Use of portion sizes containing less than 50 g available carbohydrate*

If a portion size less than 50 g available carbohydrate is to be used, then the amount of available carbohydrate in the reference food has to be reduced to be the same as that in the test food. The effect of using portions of foods containing less than 50 g available carbohydrate on the GI values obtained has not been studied. One question is whether the RGR of carbohydrates are the same at any level of carbohydrate intake. Another question is whether the variability of glycaemic responses is the same at different levels of carbohydrate intake.

Figure 2.5 shows the glycaemic responses elicited by 25, 50 and 100 g available carbohydrate doses of barley, spaghetti, instant potato, sucrose and glucose relative to a dose of white bread containing the same amount of available carbohydrate. These relative responses are calculated as F/B where F is the mean AUC elicited by the food in a group of subjects and B is the mean AUC elicted by bread; the values are not termed GI because they are not calculated using correct GI methods. Nevertheless, the mean relative responses of the five foods tested at all three levels of carbohydrate vary by less than 5%, being 91 at 25 g carbohydrate, 92 at 50 g carbohydrate and 88 at 100 g carbohydrate. This suggests that the relative responses of foods are the same at different levels of available carbohydrate intake (at least between 25 and 100 g).

The within-subject variation of glycaemic responses and its effect on the resulting GI values is discussed in detail below. In brief, increased variation of glycaemic responses not only increases the variation of the resulting GI values,

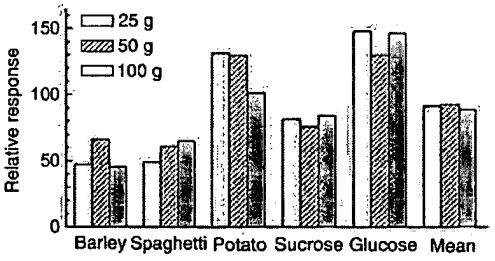

Fig. 2.5. Mean incremental AUC of blood glucose in a group of normal subjects elicited by 25, 50 and 100 g available carbohydrate doses of barley, spaghetti, instant potato, sucrose and glucose expressed as a percentage of that elicited by a dose of white bread containing the same amount of available carbohydrate. Data from Wolever and Bolognesi (1996a) and Lee and Wolever (1998).

but also skews the distribution and increases the mean of the resulting GI values. Thus, if within-subject variation of glycaemic responses, expressed as CV, increased as the portion of available carbohydrate decreased, this would have a deleterious effect on the accuracy and precision of the GI values obtained. We found recently that within-subject variation of glycaemic responses of normal subjects decreased from 98% to 28% as the dose of available carbohydrate from white bread increased from 0 to 20 g, but decreased only marginally more to 26% as the dose of available carbohydrate increased to 50 g (Table 2.4). This suggests that 20 g is the minimum amount of available carbohydrate which should be used for determining GI.

2.2.3 Method of blood sampling and glucose measurement

Capillary blood sampling was used initially for convenience and to minimize expense. Subjects can either take their own capillary blood, or samples can be taken more quickly and less invasively compared to taking blood from a forearm vein either by needle or indwelling catheter. Initially, there were concerns that measuring glucose in capillary blood may not be as precise as measuring it in venous plasma, because of the variable need to 'milk' the finger which may dilute the blood with interstitial fluid. This would be a problem if the concentration of glucose in interstitial fluid differed from that in the blood, but this is difficult to assess in practice (see Section 2.2.3.1). Regardless of this concern, there are a number of theoretical considerations, and experimental results, which suggest that measuring glucose in capillary blood is preferable to measuring it in venous plasma.

The concentration of glucose in venous plasma is less than that in arterial plasma because the tissues take up glucose from the blood as it passes from the arteries, through the capillaries to the veins (Jackson *et al.*, 1973; Coppack *et al.*, 1990). The amount of glucose taken up depends on a number of factors such as the concentration of insulin, the concentration of glucose and the subject's insulin sensitivity. The difference in concentration between arterial and venous plasma glucose concentrations (A–V difference) depends not only on the rate of uptake of glucose by tissues, but also on blood flow. For example, at a given rate of glucose uptake, reduced blood flow will result in a larger A–V difference. Venous blood is typically sampled from a forearm vein, and flow through these vessels is markedly affected by ambient temperature. Thus, it has been shown that the venous plasma glucose response to a standard meal is less at an ambient temperature of 23°C than 33°C, with blood flow being over four times greater at the higher temperature (Frayn *et al.*, 1989). Such variation in venous plasma glucose can be minimized by warming the hand in dry air at 65°C. By increasing peripheral blood flow and reducing the A–V glucose difference, this has the effect of arterializing the venous blood. Since capillary blood glucose concentration is closer to arterial, it presumably is less affected by variation in ambient temperature.

Another feature influencing venous plasma glucose differently from arterial glucose may be the fact that insulin is secreted from the normal

Table 2.4. Within-subject variation of glycaemic responses in normal subjects who took 0, 5, 10, 20, 25 or 50 g available carbohydrate portions of white bread on repeated occasions.

Dose (g)	Number of subjects	Number of repeats	Mean AUC (mmol × min/l)	Within-subject SD (mmol × min/l)	Within-subject CV (%)
0	18	4.4 ± 0.3	10.0 ± 2.4	8.3 ± 2.4	99 ± 11
5	18	4.1 ± 0.2	32.5 ± 4.4	18.8 ± 3.0	60 ± 4
10	18	4.6 ± 0.3	57.4 ± 6.3	18.9 ± 1.8	37 ± 4
20	18	4.4 ± 0.3	102.8 ± 10.6	27.4 ± 1.8	28 ± 3
25	24	4.0 ± 0.3	105.1 ± 8.0	28.1 ± 3.9	27 ± 3
50	105	7.1 ± 0.6	182.7 ± 7.1	44.8 ± 2.2	26 ± 1

Values are mean ± SEM.

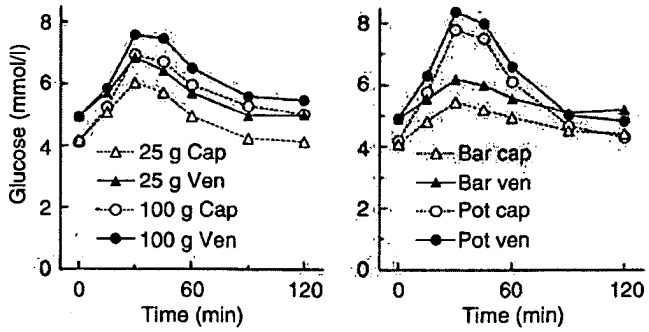

Fig. 2.6. Glucose responses in simultaneously obtained venous plasma (●,▲) and finger-prick capillary whole blood (○,△) after 25(△,▲) or 100 g (○,●) available carbohydrate from white bread (left) or 50 g available carbohydrate from pearled barley (△,▲) or instant mashed potato (○,●; right). Data from Wolever and Bolognesi (1996a).

pancreas in pulses (Matthews *et al.*, 1983) occurring at a frequency of one for every 2–3 min. Venous plasma glucose concentrations also vary up and down with a similar frequency to those of plasma insulin (Abdallah *et al.*, 1997). Presumably, therefore, the variations in plasma glucose are due to variation in tissue uptake elicited by the variable insulin concentrations. However, the glucose concentration in arterial blood would presumably vary less on a minute by minute basis than the glucose concentration in venous blood because it has not yet reached the insulin-sensitive tissues.

The glucose concentration in plasma is greater than that in whole blood because the water content of plasma is higher than that of red cells (93% vs 73%) with the glucose concentration in the water in these two compartments being identical (Burrin and Alberti, 1990). When glucose responses after different test meals were measured in simultaneously obtained venous plasma and capillary whole blood, fasting and postprandial capillary whole blood glucose concentrations were less than those in venous plasma glucose (Wolever and Bolognesi, 1996a). The difference between venous plasma and capillary whole blood glucose concentrations appears to be smaller 2 h after 100 g carbohydrate loads than after 25 or 50 g loads, but this is not the case for foods with different GI values (Fig. 2.6). The IAUC of capillary whole blood glucose was larger than that of venous plasma, and the difference appeared to vary more with amount of carbohydrate than with GI (Fig. 2.7). The random error associated with measurements of glucose in capillary whole blood was less than that associated with measurement of glucose in venous plasma for test meals consisting of single foods or mixed

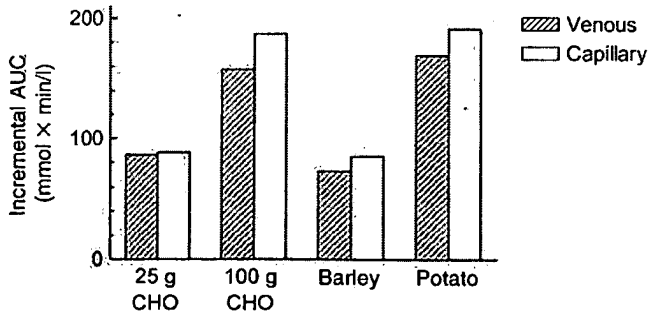

Fig. 2.7. Comparison of incremental areas under the glucose response curve measured in venous plasma or finger-prick capillary whole blood (tests shown in Fig. 2.4).

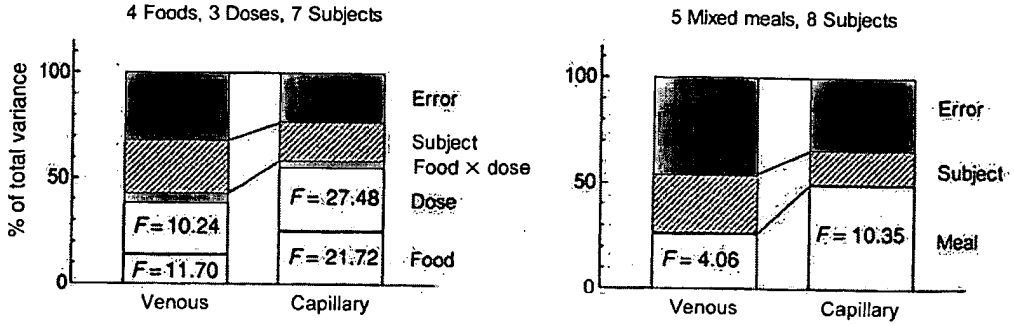

Fig. 2.8. Comparison of partitioning of variance from glucose measurements in simultaneously obtained venous plasma or capillary whole blood for individual foods (left) or mixed test meals (right). Data from Wolever and Bolognesi (1996a,b).

meals (Wolever and Bolognesi, 1996a,b) (Fig. 2.8). Therefore, differences in glycaemic response are more readily detected with capillary than venous blood sampling (Granfeldt *et al.*, 1995).

Data from an interlaboratory study also suggest that measuring glucose responses in venous plasma results in greater within-subject variation of plasma glucose responses and greater variation of GI values than measuring glucose in capillary blood (Wolever *et al.*, 2003a). In this study, five centres collected capillary blood and two collected venous blood. Each subject repeated the 50 g oral glucose trial three times, and the coefficient of variation (CV = 100 × SD/mean) of the three IAUC values after the glucose trials were calculated for each subject. The mean CV for the 47 subjects from capillary blood centres, 23.4 ± 2.1%, was significantly less than that for the 21 subjects from venous plasma centres, 56.8 ± 4.4%. The AUC values for subjects tended to be smaller from venous plasma centres, and, although the mean GI values did not differ, the variation of the GI values was much greater for the venous plasma centres (Fig. 2.9).

The site from which capillary blood is taken may affect the results. Finger-tip capillary blood responds more rapidly to changes in blood glucose than other sites. Thus, when glucose concentrations are rising rapidly after a meal, finger-prick capillary glucose concentrations are higher than capillary blood obtained from forearm, thigh or abdomen (Ellison *et al.*, 2002; Jungheim and Koschinsky, 2002; van der Valk *et al.*, 2002), whereas, when glucose concentrations are falling, finger-prick capillary glucose is lower. It is not known how blood sampling from sites other than

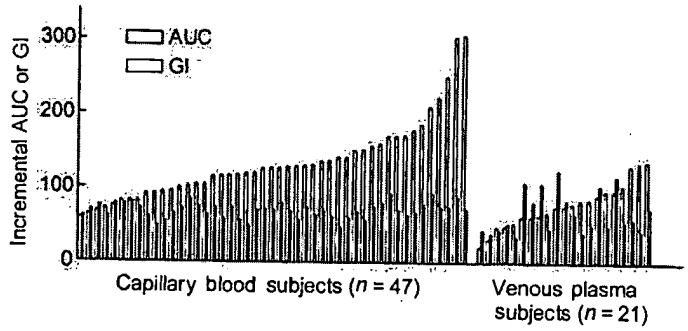

Fig. 2.9. Incremental areas under the blood glucose response curves (AUC) and mean GI values for 68 subjects who tested the GI of five different foods. Each AUC value is the mean AUC for all test foods and glucose taken by each subject, and each GI value is the mean GI of the five test foods taken by that subject. Data from Wolever *et al.* (2003a).

the finger-tip affects the IAUC and GI values of foods.

2.2.3.1 Interstitial vs blood glucose

Interstitial fluid is the water compartment which is both outside of cells and outside of the blood stream. Hormonal signals and nutrients which are carried in blood reach the target tissues only by diffusing through the interstitial fluid. Thus, the concentration of glucose in interstitial fluid, at least theoretically and in a steady-state situation, would be expected to be similar to that in the blood in nearby capillaries, but rapid changes in blood glucose are followed more slowly by interstitial glucose (Fischer *et al.*, 1987). Measurement of glucose in interstitial fluid is difficult, with microdialysis (Lonnoth *et al.*, 1987), filtration (Schmidt *et al.*, 1993) or wick (Fischer *et al.*, 1987) methods being used. Interstitial glucose concentrations have sometimes been reported to be the same as those in blood (Fischer *et al.*, 1987; Lonnoth *et al.*, 1987) and sometimes 25–50% less (Schmidt *et al.*, 1993; Sternberg *et al.*, 1995). Some of the discrepancy may be explained by the fact that insertion of the probe injures the tissue and disrupts blood flow, with low initial recoveries of glucose eventually recovering after several days (Wientjes *et al.*, 1998). Recent appreciation of the differences in capillary glucose concentrations in different sites of the body (Ellison *et al.*, 2002; Jungheim and Koschinsky, 2002; van der Valk *et al.*, 2002) may also explain some of the discrepancies, because subcutaneous interstitial glucose in abdominal adipose tissue is usually compared with plasma glucose obtained from a forearm vein.

2.2.3.2 Handling of blood samples

Red blood cells metabolize glucose, and continue to do so when blood is withdrawn from the body. Thus, if a whole blood sample is left in a tube on a bench at room temperature, the blood glucose concentration falls linearly with time. This is a potential source of measurement error, especially if tubes are left out for variable lengths of time. The fall in blood glucose can be stopped by removing the plasma or serum from contact with the cells. If one centrifuges the blood without anticoagulant, one has to wait for about 30–60 min for the blood to clot (during which time the blood glucose will fall) or else the serum removed will clot. If one wants to remove the plasma rapidly from the cells, an anticoagulant must be present in the blood collection tubes to prevent the plasma from clotting. Blood collection tubes containing a glycolytic inhibitor (such as fluoro-oxalate) are commonly used, but studies show that if such tubes are left at room temperature, glucose concentration falls for about 1 h before the inhibitor takes effect. The most reliable way to inhibit glycolysis and prevent the glucose concentration from falling is to place the sample in a refrigerator or on wet ice as soon as it has been obtained. This immediately arrests the fall in blood glucose.

2.2.3.3 Method of measuring glucose

Glucose can be measured in whole blood or plasma using various different methods. The CV of analytical variation for measuring glucose is <3% for typical clinical laboratories (Burrin and Alberti, 1990; Widjaja *et al.*, 1999). In our hands, the CV of repeated determinations of fasting glucose ($n = 100$ each measured 3 times, range 3.54–5.35 mmol/l) by YSI was 1.26% (Velangi *et al.*, 2005). In a recent interlaboratory study, three different methods of measuring glucose were used amongst the five laboratories which collected capillary blood, including: whole capillary blood glucose by glucose oxidase after mixing 1 ml 0.025 M NaOH and 50 µl 0.3 M $ZnSO_4$ solution with 50 µl whole blood, measurement of glucose in capillary plasma by hexokinase, and whole capillary blood glucose using an automatic analyser (YSI Stat 2300, Yellow Springs Instruments, OH, USA). There was no significant effect of the method of blood sampling on either the mean of variation of the GI values obtained. This suggests that any recognized and well-standardized reference method of measuring glucose is suitable for determining the GI of foods.

However, the reproducibility of dry chemistry analysers such as portable glucose metres is not as good as reference methods, with the CV of analytical variation being typically <8% (Burrin and Alberti, 1990; Widjaja *et al.*, 1999). The CV of analytical variation for various portable glucose metres ranged from 1.5% to 8.0% (Engel *et al.*, 1998; Solnica *et al.*, 2003). This degree of precision is considered adequate for the purposes of self-monitoring of blood glucose in diabetes. However, it may not be adequate for GI testing. For

reference methods of measuring glucose (CV < 3%), a glucose concentration of 5.0 mmol/l would be expected to lie between 4.7 and 5.3 mmol/l (± 6%); however, for dry methods of measuring glucose (CV < 8%), the concentration would lie between 4.2 and 5.8 mmol/l (± 16%). Since variances are additive, the variation of the incremental AUC, which is calculated from many individual glucose concentrations, would be expected to be considerably higher than the variation of each glucose measure. We recently evaluated the performance of a portable glucose meter for measuring GI of seven types of potato, and found that GI values determined by the meter were more variable than and did not agree well with those determined by YSI (Velangi *et al.*, 2005). By YSI, GI values varied from 56 ± 5 to 88 ± 8 ($P = 0.004$), but by the meter, although the range of GI values was similar (57 ± 10–81 ± 12) the means for the seven foods did not differ significantly ($P = 0.11$). This suggests that a reference method for measuring glucose should be used for determining the GI values of foods.

2.2.4 Type of subjects studied

Generally 'normal' subjects are studied, although GI values for many foods in the literature are based on tests in people with diabetes. It is generally accepted that GI values from these groups of subjects are comparable, and can be used interchangeably. However, different subjects have different degrees of within-subject variation of glycaemic responses, which influences the precision and may affect the mean of the results obtained. The implication of this is that between-subject variation of glycaemic responses does not need to be controlled when selecting subjects for determining the GI of foods, but the degree of within-subject variation may be important to consider. In an attempt to obtain a homogeneous group of subjects, investigators may tend to select young, fit subjects with no family history of diabetes for glycaemic response studies. Ironically, this type of subject may not be ideal for determining GI values because they will tend to be insulin-sensitive with low glycaemic responses, and day-to-day variation in response which is relatively high in relation to the mean.

2.2.4.1 *Mean GI values in different subjects*

For most foods and most subjects, mean GI values are the same in subjects with different degrees of carbohydrate intolerance. This is based on the results of studies such as those shown in Fig. 2.10 which show strong correlations between GI values determined in normal subjects and subjects with diabetes (Fig. 2.10A), subjects with type 2 diabetes and subjects with type 1 diabetes (Fig. 2.10B) and European and African subjects (Fig. 2.10C). In addition, the mean of the GI values for the 21 foods determined in normal subjects, 78 ± 6, was not significantly different from that in subjects with diabetes, 74 ± 6, and the mean of the GI values in the European subjects, 54 ± 5, was the same as that in the African subjects,

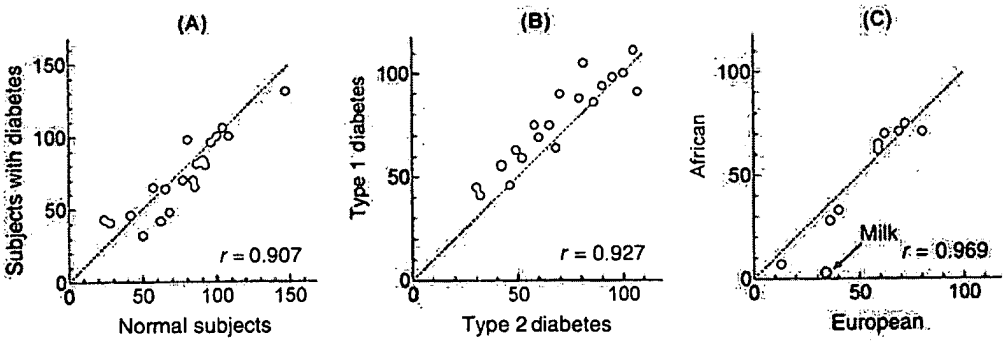

Fig. 2.10. Correlations between GI values for the same foods measured in normal and diabetic subjects (A), subjects with type 2 diabetes and subjects with type 1 diabetes (B) and normal European and normal African subjects (C). Dotted lines = lines of identity. Data from Jenkins *et al.* (1981a, 1983), Wolever *et al.* (1987) and Walker and Walker (1984).

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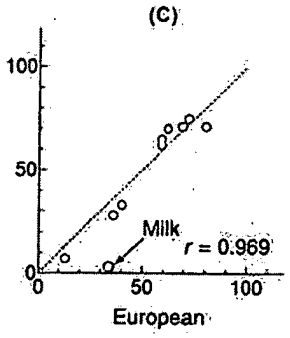

normal and diabetic subjects
normal European and normal
(1981a, 1983), Wolever *et al.*

54 ± 5, with the exception of milk. Milk had a lower GI in Africans than in Europeans, and the mean of the GI values for 20 foods tested in subjects with type 1 diabetes, 76 ± 5, was significantly greater than that for the same foods tested in subjects with type 2 diabetes, 68 ± 5 ($P = 0.001$). The example of milk illustrates the only situation where major differences in GI between subjects may occur, that is, when the efficiency of absorption of the test food is relatively different than that of the reference food. Africans and Europeans absorb glucose and starch to the same extent, but Africans have a much higher prevalence of low intestinal lactase activity than Europeans, so the Africans would absorb the lactose in milk to a lesser extent than the Europeans. The small difference in GI between subjects with type 1 and type 2 diabetes can be explained by the fact that within-subject variation differs in these subject groups.

2.2.4.2 Day-to-day variation of glycaemic responses in different subjects

Day-to-day (or within-subject) variation of glycaemic responses differs markedly in different groups of subjects. Within-subject variation is assessed by having the subject repeat exactly the same test meal (usually the reference test meal) on different days. Early on we assessed the day-to-day variation of glycaemic responses in subjects who were normal, or who had type 2 diabetes treated with diet or oral agents, type 2 diabetes treated with insulin, or type 1 diabetes (Wolever *et al.*, 1985) (Table 2.5). This showed that the average within-subject CV (CV = 100 × SD/mean) in subjects with type 2 diabetes, 15%, was significantly less than that in subjects with type 1 diabetes, 29%, and normal subjects having an intermediate value. Rasmussen *et al.* (1992) determined the variation of plasma glucose and insulin responses in ten subjects with type 2 diabetes who repeated trials of white bread three times each. They obtained a mean CV for plasma glucose within subjects of 19%, similar to our value of 15%, but a much higher CV for plasma insulin, 41%. More recently, we determined the reproducibility of plasma glucose and insulin responses in lean and obese normal subjects, subjects with IGT and subjects with type 2 diabetes after 75 g oral glucose and a solid mixed test meal 'bar' (Wolever *et al.*, 1998a,b). Reproducibility after glucose was similar to that after the bar (Table 2.6). This study confirmed that subjects with IGT and type 2 diabetes tended to have lower within-subject variation than normal subjects, significantly so after glucose (12% in subjects with diabetes vs 39% in lean normal subjects). However, in contrast to Rasmussen *et al.* (1992), the CV values for insulin (19–29%) were similar to those for glucose (12–39%) and did not differ between different subject groups (Table 2.6).

Differences in within-subject variation are important, because, as shown above (Fig. 2.1), most of the variation of GI values is due to within-subject variation. Because the GI is a ratio of two independently variable glycaemic response AUC values, not only is the variation of GI

Table 2.5. Between- and within-subject variation in glycaemic responses in different groups of subjects who took 50 g glucose (normal subjects) or 50 g available carbohydrate portions of white bread (subjects with diabetes) on repeated occasions.

Subject group	*n* (range)	Mean (mmol × min/l)	$CV_{between}$ (%)	SD (mmol × min/l)	CV_{within} (%)
Normal ($n = 11$)	8.3 (4–15)	208 ± 55[a] (129–290)	26.4	57 ± 40[a] (14–136)	25.0 ± 12.0[ab] (9.0–46.9)
Type 2 not on insulin ($n = 10$)	7.8 (5–11)	658 ± 222[b] (291–1045)	33.7	96 ± 38[a] (50–167)	15.9 ± 7.1[ab] (5.6–27.1)
Type 2 on insulin ($n = 12$)	10.6 (6–27)	968 ± 224[c] (611–1373)	23.1	139 ± 41[a] (89–205)	14.8 ± 4.4[a] (8.6–22.1)
Type 1DM ($n = 14$)	9.4 (5–35)	1066 ± 358[c] (555–1658)	33.6	268 ± 134[b] (78–640)	29.1 ± 19.8[b] (7.1–71.3)

[abc]Means in a column with different letter superscripts differ significantly, $P < 0.05$. Values are mean ± SD with the range in brackets.
Data from Wolever *et al.* (1985).

Table 2.6. Mean and within-subject variation of incremental areas under the plasma glucose and insulin response curves in different groups of subjects who took 75 g glucose 4 times and 50 g available carbohydrate from a mixed solid test meal wafer four times.

	Mean (mmol × min/l)		CV (%)	
	GTT	BAR	GTT	BAR
Plasma glucose				
Normal lean ($n = 10$)	215 ± 44[a]	108 ± 24[a]	38.7 ± 6.1[a]	30.6 ± 6.3
Normal obese ($n = 9$)	268 ± 36[a]	106 ± 13[a]	26.0 ± 5.9[ab]	32.5 ± 4.6
IGT ($n = 9$)	537 ± 26[b]	276 ± 19[b]	18.9 ± 2.7[ab]	16.5 ± 3.7
Type 2 diabetes ($n = 8$)	803 ± 59[c]	435 ± 43[c]	11.6 ± 1.8[b]	18.1 ± 1.6
Plasma insulin				
Normal lean ($n = 10$)	15.2 ± 2.0	9.4 ± 1.4	20.6 ± 2.4	19.7 ± 3.4
Normal obese ($n = 9$)	24.2 ± 4.6	11.2 ± 1.7	29.0 ± 5.0	21.2 ± 2.6
IGT ($n = 9$)	35.6 ± 7.4	23.2 ± 4.6	19.5 ± 3.1	23.7 ± 4.0
Type 2 diabetes ($n = 8$)	20.6 ± 5.6	16.1 ± 3.7	18.8 ± 4.0	20.2 ± 4.1

Values are mean ± SD with the range in brackets.
[abc]Means in a column with different letter superscripts differ significantly, $P < 0.05$.
Data from Wolever *et al.* (1988a,b).

values greater than the within-subject variation of the glycaemic responses, but also increasing within-individual variation of glycaemic responses skews the distribution of the GI values and increases the mean (Wolever *et al.*, 1991a).

Absolute glycaemic responses of normal subjects are not normally distributed because of differences in response between subjects (left side of Fig. 2.11). However, within subjects, the responses are normally distributed as illustrated in the right side of Fig. 2.11. The data shown here represent 720 individual tests of 50 g available carbohydrate portions of white bread taken by 98 subjects each of whom tested bread at least three times (range 3–35, median 5). The left side of Fig. 2.11 shows that the normalized AUC values (each AUC was expressed as a Z score, i.e. the number of SDs from the mean of the same subject) are normally distributed. The same is true for subjects with diabetes (Wolever, 1990b). Thus, mathematical

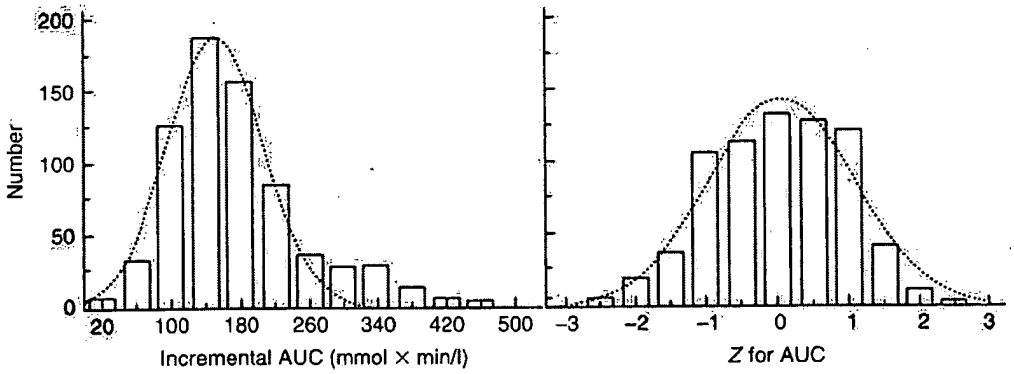

Fig. 2.11. Distribution of glycaemic response (incremental area under the curve) elicited by 50 g available carbohydrate portions of white bread. Data represent 720 individual tests of bread taken by 98 normal subjects with each subject doing at least three tests (range 3–35). Bars represent numbers of subjects with the value being the centre of the bin. Dotted lines represent the normal distribution with the same mean and SD. Left: distribution of AUC values is significantly skewed. Right: distribution of Z scores is not significantly different from the normal distribution.

modelling using the normal distribution can be used to illustrate the effects of altering the variation of glycaemic responses on the GI values obtained. Table 2.7 shows examples of GI calculations with within-subject CV of glycaemic responses being either 30% (representing subjects with type 1 diabetes) or 15% (representing subjects with type 2 diabetes). In addition the effect of using the average of three trials of the reference food to calculate the GI is illustrated. If C equals the within-subject CV of glycaemic response AUCs elicited by the reference food, then $C/\sqrt{3}$ equals the CV of the average of three trials of the reference food. Thus, in Table 2.7, the CVs of the reference food have been reduced by a factor of $\sqrt{3} = 1.73$. Assuming a normal distribution,

Table 2.7. Model of effect of altering day-to-day variation of glycaemic responses on GI values.

a. CV = 30%; one trial of reference food.

		−2SD (−60%)	−1SD (−30%)	Test mean	+1SD (+30%)	+2SD (+60%)
Deviation	Area	360	630	900	1170	1440
−2SD (−60%)	480	75	131	188	244	300
−1SD (−30%)	840	43	75	107	139	171
Reference mean	1200	30	53	75	98	120
+1SD (+30%)	1560	23	40	58	75	92
+2SD (+60%)	1920	19	33	47	61	75

b. CV = 30%; average of three trials of reference food.

		−2SD (−30%)	−1SD (−15%)	Test mean	+1SD (+15%)	+2SD (+30%)
Deviation	Area	360	630	900	1170	1440
−2SD (−34.6%)	784	46	80	115	149	184
−1SD (−17.3%)	992	36	64	91	118	145
Reference mean	1200	30	53	75	98	120
+1SD (+17.3%)	1408	26	45	64	83	102
+2SD (+34.6%)	1616	22	39	56	72	89

c. CV = 15%; one trial of reference food.

		−2SD (−30%)	−1SD (−15%)	Test mean	+1SD (+15%)	+2SD (+30%)
Deviation	Area	630	765	900	1035	1170
−2SD (−30%)	840	75	91	107	123	139
−1SD (−15%)	1020	62	75	88	102	115
Reference mean	1200	53	64	75	86	98
+1SD (+15%)	1380	46	55	65	75	85
+2SD (+30%)	1560	40	49	58	66	75

d. CV = 15%; average of three trials of reference food.

		−2SD (−30%)	−1SD (−15%)	Test mean	+1SD (+15%)	+2SD (+30%)
Deviation	Area	630	765	900	1035	1170
−2SD (−17.3%)	992	64	77	91	104	118
−1SD (−8.7%)	1096	58	70	82	94	107
Reference mean	1200	53	64	75	86	98
+1SD (+8.7%)	1304	48	59	69	79	90
+2SD (+17.3%)	1408	45	54	64	74	83

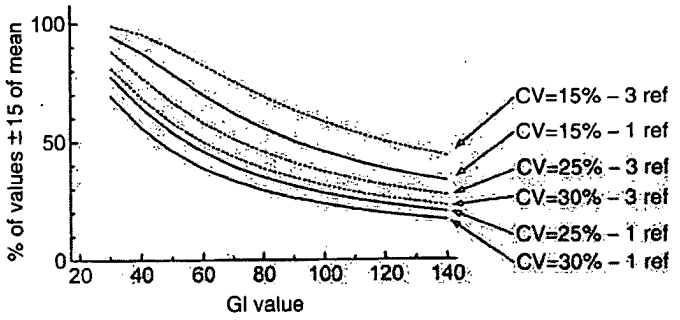

Fig. 2.12. Estimated percentage of subjects whose individual GI values will lie within a range of ± 15 from the true GI for different values of GI, for different degrees of within-subject variation of AUC values (CV = coefficient of variation, 30% represents subjects with type 1 diabetes, 25% represents normal subjects and 15% represents subjects with type 2 diabetes), and for GI values calculated using either one repeat of the reference food (1 ref) or three repeats (3 ref). Data derived from mathematical modelling (see text).

approximately 47% of all GI values fall within the range of the nine GI values outlined by the bold lines (AUC for both reference and test foods within ± 1 SD of mean). Approximately 44% of all GI values fall between the inner 9 and outer 16 GI values (AUC for either reference or test food more >1 SD but <2 SD from the mean). Approximately 9% of all GI values fall outside the range of all 25 GI values on each table (AUC for either reference or test food >2 SD from the mean). The range of GI values in the example representing type 1 diabetes with only one test of the reference food goes from 19 to 300, which is 25–400% of the true value. Using the mean of three reference tests reduces the range of GI values obtained from 29% to 245% of the true value. Using a CV of 15%, representing subjects with type 2 diabetes, and the mean of three trials of the reference food, yields a range of GI values from 60% to 157% of the true value.

A grid similar to that shown in Table 2.7 was created, but with deviations going from −3 to +3 SDs in steps of 0.5 SDs. Assuming a normal distribution, the average GI value and the likelihood of obtaining a value in that cell was calculated. This allows one to estimate the theoretical effect of changing the mean and CV of AUC values for the reference and test foods on the distribution and mean GI of the values obtained. Figure 2.12 shows the estimated % of subjects whose individual GI values will lie within a range of ± 15 from the true GI for different values of GI (mean AUC for theoretical test food expressed as a percentage of the mean AUC for the theoretical reference food) and for different degrees of within-subject variation of AUC values. The percentage of GI values within ± 15 from the true mean increases as the within-subject CV is reduced and also as the GI value is reduced. Figure 2.13 shows the observed proportion (± 95% CI) of 47 normal subjects with a GI value ± 15 from the mean for five foods with GI ranging from 35 to 91 (Wolever *et al.*, 2003a), plotted against the expected proportion from Fig. 2.12. The relatively good correlation suggests that the type of mathematical modelling illustrated in Table 2.7 and Fig. 2.12 is valid.

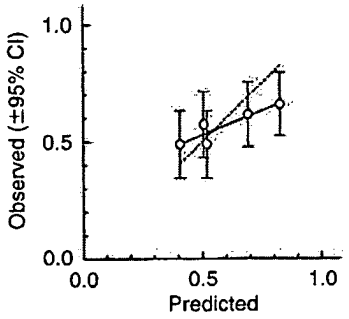

Fig. 2.13. Observed (with 95% confidence interval, CI) vs predicted proportion of 47 subjects with an individual GI value within ± 15 of the mean for five foods with GI ranging from 35 to 91. Predicted proportion derived from Fig. 2.12, observed proportion derived from Wolever *et al.* (2003a). Dotted line = line of identity; solid line = regression line.

Theoretical consideration of the effects of increased random variation of glycaemic responses on GI values also suggests that as the variation increases, the mean GI increases. The magnitude of the effect predicted by the model discussed above is shown in Fig. 2.14, which shows how the difference in GI (theoretical observed minus true) increases both as day-to-day variation of glycaemic responses increases and as the GI itself increases. Thus, it would be predicted that subjects with high within-subject variation, such as those with type 1 diabetes, would have somewhat higher GI values than subjects with low within-subject variation, such as those with type 2 diabetes. Figure 2.10B shows that GI values determined in subjects with type 1 diabetes are slightly, but significantly, higher than GI values determined in subjects with type 2 diabetes. The magnitude of the observed difference, 7.7, is greater than that expected by the model shown in Fig. 2.14, but this may be explained by the fact that the model calculations include the entire population of subjects; whereas the real data are drawn from a sample of the real population which reduces the precision of the observed means.

2.2.4.3 *Effect of preprandial glucose on postprandial glycaemic response*

Early on, we noted that the incremental area under the blood glucose response curve of subjects with type 1 diabetes was inversely related to their FBG concentration immediately preceding the test meal (Jenkins *et al.*, 1984); that is the higher FBG, the lower the incremental area under the blood glucose response curve (AUC). Later we reported that the same phenomenon tended to occur in normal subjects and those with type 2 diabetes although the strength of the relationships were much less than in subjects with type 1 diabetes (Wolever *et al.*, 1985). Others have noted a similar phenomenon (Nielsen and Nielsen, 1989) but suggested that the correlation between FBG and AUC was apparent only when FBG >13 mmol/l. However, our data do not support this. Figure 2.15 shows data for 205 trials of reference white bread done by 18 subjects with type 1 diabetes, and 931 trials of reference white bread done by 54 subjects with type 2 diabetes. The solid dots represent trials for which fasting glucose was <13.0 mmol/l, and the open circles represent trials for which fasting glucose was ≥13.0 mmol/l. For subjects with type 1 diabetes, FBG was significantly related to AUC when FBG <13 mmol/l, $r = -0.318$ ($P < 0.001$); when FBG ≥13 mmol/l, there was a strong trend for a negative correlation, $r = -0.263$, which missed statistical significance ($P = 0.092$). Similarly, for subjects with type 2 diabetes, there was a weak correlation when FBG < 13 mmol/l, $r = -0.106$, which was statistically significant because of the large number of points ($P = 0.002$); with the correlation when FBG ≥ 13 mmol/l being stronger ($r = -0.163$), but not statistically significant ($P = 0.12$). The regression lines for AUC on FBG had almost identical slopes and y-intercepts in subjects with both type 1 and type 2 diabetes when FBG < 13 or ≥13 mmol/l (Fig. 2.15). These data suggest that the negative correlation between FBG and AUC is the same across the entire range of FBG, but is less strong (i.e. the

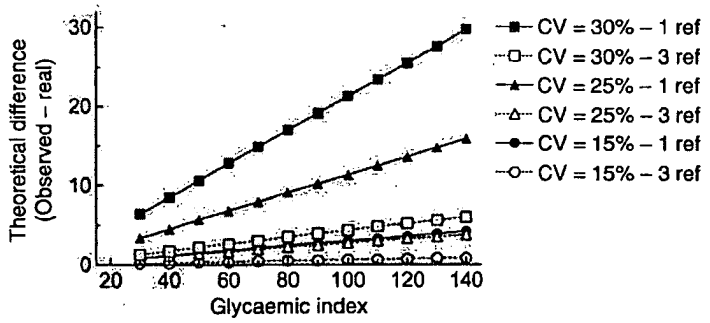

Fig. 2.14. Theoretical effect of increased day-to-day variation (CV) and number of reference foods used on the difference in GI (observed minus real) based on mathematical modelling.

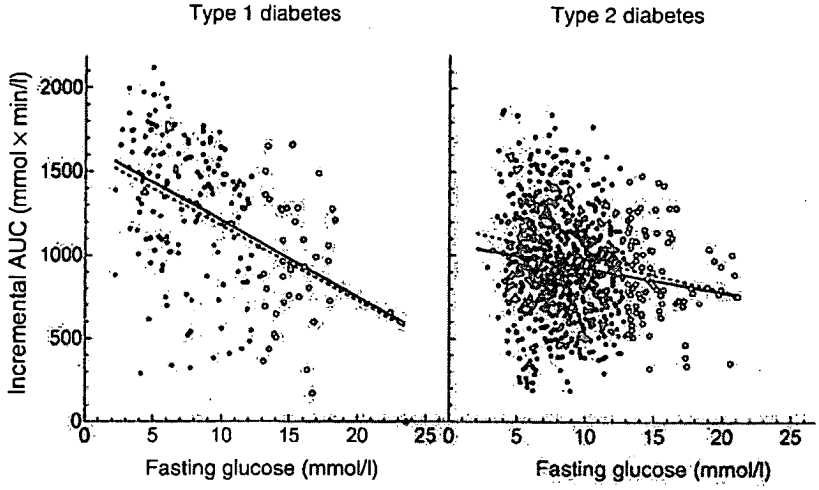

Fig. 2.15. Relationship between fasting glucose and incremental area under the curve for repeated tests of white bread taken by subjects with type 1 diabetes (left) and type 2 diabetes (right). Solid lines = regression lines for fasting glucose <13 mmol/l (filled circles); dotted lines = regression lines for fasting glucose >12.9 mmol/l (open circles). See text for further description.

slope is less) in people with type 2 diabetes than in those with type 1 diabetes.

Table 2.8 shows mean data for repeated trials of white bread to compare the relationships between FBG and AUC in 18 individual subjects with type 1 diabetes compared to 54 subjects with type 2 diabetes each of whom tested white bread at least three times. The subjects with type 1 diabetes did an average of 11 trials of bread each, compared to 17 for subjects with type 2 diabetes (ns). Although mean FBG did not differ significantly between type 1 and type 2 subjects, the variation of FBG in subjects with type 1 diabetes was more than twice that in subjects with type 2 diabetes ($P < 0.001$). Mean AUC was significantly higher in subjects with type 1 dia-

Table 2.8. Within-subject relationships between fasting glucose and incremental area under the curve elicited by test meals of white bread containing 50 g available carbohydrate taken repeatedly by subjects with type 1 and type 2 diabetes.

	Type 1	Type 2	P*
Number of subjects	18	54	
Number of repeated trials per subject	11.4 ± 3.0	17.2 ± 1.9	ns
Fasting glucose (mmol/l)	9.4 ± 0.6	8.9 ± 0.3	ns
SD of fasting glucose (mmol/l)[a]	3.9 ± 0.4	1.8 ± 0.1	<0.001
CV of fasting glucose (%)[a]	43.5 ± 3.7	20.2 ± 1.4	<0.001
IAUC (mmol × min/l)	1109 ± 81	876 ± 33	0.002
SD of IAUC (mmol × min/l)[a]	278 ± 27	155 ± 8	<0.001
CV of IAUC (%)[a]	28.0 ± 4.0	18.3 ± 0.9	<0.001
Adjusted CV of IAUC (%)[b]	19.2 ± 3.1	15.6 ± 0.9	ns
Regression slope (min)[c]	−48.8 ± 6.5	−17.2 ± 9.9	0.078
Regression *y*-intercept (mmol × min/l)[c]	1115 ± 80	877 ± 33	0.002
Correlation coefficient (*r*)[c]	−0.634 ± 0.063	−0.164 ± 0.060	<0.001
n (%) with significant *r*-value[c]	8 (44%)	7 (13%)	0.004

*Significance of difference between Type 1 and Type 2; ns=not significant.

[a]SD=standard deviation; CV=coefficient of variation (100 × SD/mean); IAUC=incremental area under the blood glucose response curve.

[b]CV of residuals; i.e. differences between individual IAUC values and estimates of IAUC at the same fasting glucose from the regression equation of IAUC on fasting glucose.

[c]Slope, y-intercept, correlation coefficient and significance of correlation coeffcient for regression of IAUC on fasting glucose.

betes by about 26%, but the variation in AUC in subjects with type 1 diabetes was almost twice that in subjects with type 2 diabetes ($P < 0.001$). The correlation coefficients and regression equations between FBG and AUC were calculated for each individual subject. Although the slope of the regression line in type 1 subjects was nearly three times that in type 2 subjects, because of high variation in the type 2 subjects, the difference did not reach statistical significance ($P = 0.078$). However, the mean correlation coefficient in type 1 subjects was nearly four times that in type 2 subjects (−0.634 vs −0.164, $P < 0.001$), and the correlation between FBG and AUC was significant in 44% of the type 1 subjects compared to only 13% of the type 2 subjects ($P = 0.004$). If the individual correlations between FBG and AUC are used to adjust the AUC values for the variation in FBG and the variability of the adjusted AUC values is calculated, one finds that the mean CV (CV = 100 × SD/mean) in type 1 subjects is reduced by nearly 50% to 19.2%, a value which is not longer significantly different from the mean CV of the adjusted AUC values in the type 2 subjects, 15.6% (Table 2.8).

Figure 2.16 shows the correlations between FBG and AUC in the eight subjects with the largest number of white bread trials. All the subjects whose data are shown in Fig. 2.16 had type 2 diabetes, except subject #2, who had type 1 diabetes. These data illustrate that the variation in FBG is less in most of the subjects with type 2 diabetes than the subject with type 1 diabetes, and that the slopes of the regression lines and degree of significance of the correlation coefficients are less in the subjects with type 2 than type 1 diabetes. Thus, part of the reason for the lack of correlation between FBG and AUC in type 2 diabetes is the smaller range of variation in FBG and AUC in type 2 subjects. However, this is not the whole story. Subject 1 (type 2 diabetes) has the same range in FBG as subject 2 (type 1 diabetes), but the correlation coefficient is much less. All of these facts shed some light as to why incremental AUC is reduced when FBG is high.

One potential reason why incremental AUC gets smaller as FBG increases is because of more and more glucose being lost in the urine as the blood glucose increases. However, if loss of glucose into the urine were the only reason, then the slope of the regression line for AUC on FBG should be the same in subjects with type 1 and type 2 diabetes, particularly those with a high range of variation of FBG, such as subjects #1 and #2. The fact that this is not so suggests that other factors must be playing a role, and a likely factor is what is termed 'glucose effectiveness', i.e. the ability of plasma glucose to stimulate its own removal. Glucose enters cells via different types of glucose transporters of which more than a dozen different types are known (Wood and Trayhurn, 2003). Factors which increase the transport activity of glucose transporters include glucose, insulin and exercise (Khayat *et al.*, 2002; Koistinen and Zierath, 2002). The most well-known transporters are the class I facilitative transporters GLUT1–4. GLUT1–3 transporters are not sensitive to insulin, that is, the rate at which they transport glucose is not affected by the insulin concentration. GLUT2 is primarily expressed in liver, pancreas, intestine and kidney and is believed to be part of the glucose sensing mechanism. GLUT3 is primarily expressed in brain. GLUT1 is expressed in all tissues, and particularly in brain and red blood cells, but also in muscle and fat. GLUT1, therefore, is a mechanism by which muscle can take up glucose in the absence of insulin at a rate which depends on the concentration of glucose in the blood. Insulin-stimulated glucose uptake is primarily mediated by GLUT4. A rise in plasma insulin increases glucose transport by causing GLUT4 transporters to be translocated from vesicles in the cytoplasm of cells to the surface of cells, and by increasing the intrinsic glucose transport activity of the transporters themselves (Furtado *et al.*, 2002).

2.2.5 Type of reference food

The 'reference food' is the carbohydrate source against which other foods are compared, and, by definition, has a GI of 100. The original reference food was glucose (Jenkins *et al.*, 1981a), but later, when we started doing tests mainly in subjects with diabetes, we used whole meal bread (Jenkins *et al.*, 1983). The rationale for switching was because bread was considered a more physiological carbohydrate source. It was felt that the high osmolarity of oral glucose solutions may delay gastric emptying and/or produce nausea in some individuals which, in turn, might make glycaemic responses more variable (Thompson *et al.*, 1982), as well as being unpleasant for the subjects. Since the whole meal bread produced the same glycaemic response as white bread, we began using white bread as the reference food. This raises

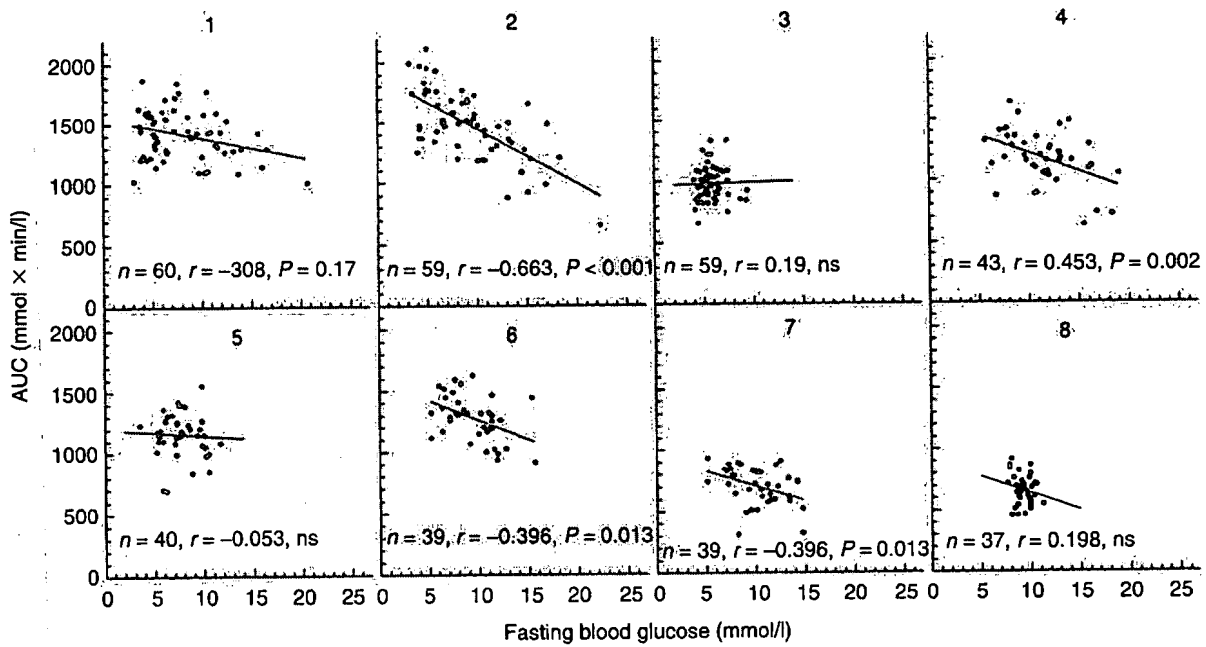

Fig. 2.16. Relationship between fasting blood glucose and incremental area under the blood glucose response curve elicited by 50 g available carbohydrate from white bread in eight individual subjects with diabetes. Subject 2 had type 1 diabetes, all the others had type 2 diabetes. Lines are regression lines.

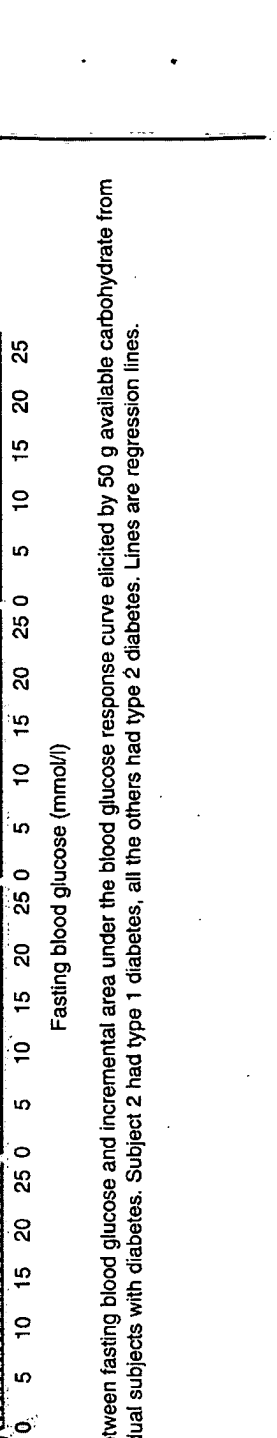

Fig. 2.16. Relationship between fasting blood glucose and incremental area under the blood glucose response curve elicited by 50 g available carbohydrate from white bread in eight individual subjects with diabetes. Subject 2 had type 1 diabetes, all the others had type 2 diabetes. Lines are regression lines.

two questions. Is bread better than glucose as a reference food for GI testing? Since GI values based on different reference foods differ; is it valid to convert GI values based on one reference food to those based on another?

Subjects may prefer eating bread rather than glucose, and this is one reason why bread may be preferred as a reference food. If glycaemic responses elicited by bread were less variable than those elicited by glucose, this would be a strong reason to use bread instead of glucose. However, there is no evidence that day-to-day variation of glycaemic responses when subjects take repeated tests of glucose differs from that for bread. Table 2.5 shows that, in 11 normal subjects, the mean ($\pm$ SEM) CV within subjects for repeated tests of 50 g glucose was 25 $\pm$ 4% (Wolever *et al.*, 1985), and in 47 subjects who repeated trials of 50 g oral glucose two to three times in the interlaboratory GI study, mean CV was 23.4 $\pm$ 2.1% (Wolever *et al.*, 2003a). For bread, the mean within-subject CV was 27.7 $\pm$ 5.0% in ten subjects who repeated bread trials three times each (Wolever *et al.*, 2003a) and 20.4 $\pm$ 1.8% in 13 subjects who repeated bread trials four times each. The fact that the average CV for glucose, 24%, is virtually identical to that for bread, 24%, suggests that, at least from a technical point of view, either are equally good reference foods for determining the GI of foods.

GI values for foods based on bread as a reference (GI_{wb}) are higher than those based on glucose (GI). Therefore, when reading the literature on GI it is important to know what the reference food is. I showed in my PhD thesis that the GI_{wb} values could be converted to GI values by simply dividing the GI_{wb} values by $100/x$ where x is the mean GI value of white bread. In my studies the mean GI of white bread was 75, and so GI_{wb} values were adjusted by dividing by 1.333. The mean GI value of the 21 foods, 58.52 $\pm$ 4.52, was virtually identical to the mean of the adjusted GI_{wb} values, 58.43 $\pm$ 4.65. The linear regression of the adjusted GI_{wb} values on GI values produced a regression line which was virtually superimposed on the line of identity across the entire range of GI values (Fig. 2.17). This showed that it is valid to adjust GI values based on reference foods other than glucose. The adjustment factor from my PhD thesis, 1.333, was less than that recommended by Dr Jennie Brand-Miller, 1.4. But this is probably because I used 50 g dextrose in my PhD thesis. Dextrose is glucose monohydrate which has a molecular weight 10% more than that of anhydrous glucose. Thus, to obtain 50 g anhydrous glucose, 55 g of dextrose is required. Increasing my conversion factor of 1.333 by 5% yields a factor of exactly 1.4! In the interlaboratory GI study, 50 g anhydrous glucose was the reference food, and the mean GI of white bread tested in 47 subjects was 71.0, resulting in a conversion factor of 1.408.

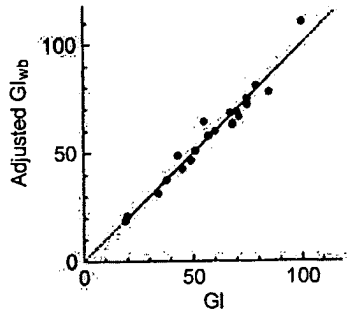

Fig. 2.17. Relationship between glycaemic index calculated using glucose as the reference food (GI) and glycaemic index calculated using white bread as the reference food (GI_{wb}) and adjusted by multiplying the GI_{wb} values by $W/100$, where W = the GI of white bread (glucose = 100). Data are from groups of normal subjects each of whom consumed both glucose and white bread. Dotted line = line of identity, solid line = regression line. Data from Wolever (1986).

It is not ideal to have two different sets of GI values in the literature. Therefore, it is recommended that GI values be expressed on the glucose scale. It is valid to use bread or some other food as the reference, but the results should be adjusted to the glucose scale. If a reference food other than glucose is to be used, then the GI value of the reference food relative to glucose should be well characterized and periodically checked to ensure that an accurate conversion factor is used.

2.2.6 Time of day tests are done

Blood glucose responses vary throughout the day depending on the time of day (Malherbe *et al.*, 1969; Genuth, 1973; Service *et al.*, 1983; Wolever and Bolognesi, 1996c), the nature of the meal consumed (Service *et al.*, 1983; Daly *et al.*, 1998), the nature of the previous meal consumed (Jenkins *et al.*, 1982b; Collier *et al.*, 1987; Wolever *et al.*, 1988a, 1995a; Nestler *et al.*, 1988; Liljeberg and

Björck, 2000) and intermeal spacing (Staub, 1921; Trougott, 1922). These studies suggest that absolute glycaemic responses may vary at different times of the day. However, it is generally assumed that the relative effects of meal components on glycaemic responses is the same; i.e. if a change in the nature of the test meal has a certain effect at breakfast, the relative effect will be the same at lunch or dinner.

Most glycaemic response tests are done in the morning after an overnight fast, but some have been done at lunch time after a standard breakfast and the results used to draw general conclusions about the validity of the GI (Coulston *et al.*, 1984a, 1987) or about the effects of varying meal size and composition (van Amelsvoort *et al.*, 1990a,b). There is evidence that treatments which influence blood glucose responses at breakfast and dinner may not have such large effects at lunch. For example, in a study of low-GI diets in healthy subjects, Jenkins *et al.* (1987a), glycaemic responses were lower after low-GI breakfast and dinner meals, but the expected difference at lunch was not seen. Similarly, in subjects with type 2 diabetes, there was no difference in blood glucose after a low-GI lunch, but the expected difference was seen after breakfast (Brand *et al.*, 1991). In addition, in subjects with IGT, the α-glucosidase inhibitor acarbose reduced glucose and insulin responses to a lesser extent after lunch than after dinner or breakfast, despite the same dose being taken (Chiasson *et al.*, 1996). On the other hand, this is not a universal effect, with the expected reductions in glucose and/or insulin being observed at lunch in some studies (Hollenbeck *et al.*, 1988; Järvi *et al.*, 1999).

One problem with interpreting these studies is that the test meals fed at breakfast, lunch and dinner were not the same. Thus, it is not really possible to know if the difference in relative effects seen at different times of the day was really due to diurnal variation or simply to differences in the meals. We did a study in eight normal subjects to see if the relative glycaemic effects of two breakfast cereals were the same when fed in the morning after an overnight fast, or at mid-day, exactly 4 h after starting to eat a standard breakfast consumed under supervision in the laboratory (Wolever and Bolognesi, 1996c). As expected, when consumed at breakfast after an overnight fast, the high-fibre, oat loop cereal (GI = 61) elicited a glycaemic response almost exactly 50% of that elicited by a portion of maize flakes (GI = 124) containing the same amount of available carbohydrate (Fig. 2.18), but there was no difference in glycaemic response between the cereals when consumed at mid-day, 4 h after a standard breakfast. It is not known if time of day affects the RGR in other groups of subjects, such as those with insulin resistance or diabetes. In addition, our results do not indicate whether the lack of difference at lunch was due to the time of day *per se*, or to the fact that a meal had been consumed 4 h previously. Nevertheless, the results suggest that time of day may need to be taken into account when interpreting the literature. In addition, they are the basis for my recommendation that studies done for the purpose of determining the GI of foods should be done in the morning after an overnight fast.

It is of interest that diurnal variation affects not only blood glucose and insulin responses, but also chylomicron–triglyceride responses. When a single fat-rich test meal is fed in the morning after an overnight fast, serum triglyceride concentrations peak about 3–5 h after the meal. However, when a second meal is consumed 4–6 h after the first one, there is more rapid appearance of chylomicrons into the circulation, with a peak in serum triglycerides about 1 h after the meal (Wolever, 1990a; Fielding *et al.*, 1996). It has been suggested that the early postprandial peak in chylomicrons is due to the fact that a propor-

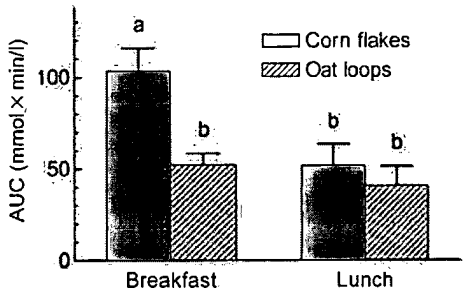

Fig. 2.18. Mean ± SEM incremental areas under the glycaemic response curve (AUC) of eight normal subjects after consuming equal-available carbohydrate portions of corn flakes or oat loops either in the morning after 10–14 h overnight fasts (breakfast) or 4 h after a standard breakfast (lunch). Means with different letter superscripts differ significantly ($P < 0.05$). Data from Wolever and Bolognesi (1996c).

tion of the fat in the first meal remains in the intestine or the enterocytes and enters the circulation with ingestion of the second meal (Fielding *et al.*, 1996). The early postprandial peak is similar whether the second meal is rich in fat or carbohydrate (Evans *et al.*, 1998). In contrast to the reduced difference in glycaemic response between carbohydrate foods fed at lunch, the difference in blood levels of different fatty acids appears to be maintained or even enhanced after the second meal (Jackson *et al.*, 2002).

2.2.7 Preparation of subjects before the test day

The glycaemic responses elicited by the reference test meal vary from day-to-day within subjects. Since most of the variation in GI values is due to within-individual variation (Fig. 2.1), the precision of GI results can be improved by procedures which reduce within-subject variation. The use of capillary as opposed to venous blood sampling is associated with reduced within-subject variation (Figs 2.8 and 2.9), as is the use of the mean AUC of three trials of the reference test in each subject (Table 2.7). Factors such as stress (Surwit *et al.*, 1992; Armario *et al.*, 1996), recent exercise (Mikines *et al.*, 1988; Langfort *et al.*, 1991; Borghouts and Keizer, 2000), cigarette smoking (Frati *et al.*, 1996), length of fasting time (Berkus *et al.*, 1990; Horton and Hill, 2001; Emberson *et al.*, 2002) and previous meal (Jenkins *et al.*, 1982b; Collier *et al.*, 1987; Nestler *et al.*, 1988; Wolever *et al.*, 1988a, 1995a; Liljeberg and Björck, 2000) have been shown to affect carbohydrate metabolism. Thus, variation in these factors may increase day-to-day variation in blood glucose responses. In an attempt to control some of these factors, some investigators provide subjects with a standard dinner to be consumed at a set time, the evening before a glycaemic response test, and ask them not to smoke or to do any vigorous physical activity for 24 h before the test (Wolf *et al.*, 2001a,b). These steps add cost to the study and make it more difficult for subjects to comply with the experimental protocol, and, at the time, it was not known whether this actually reduced day-to-day variation. Therefore, we did a study to see whether within-individual variation of glycaemic responses to a standard breakfast test meal could be reduced by controlling subjects' dinner, activities, stress and fasting time for 24 h prior to the test.

Thirteen healthy subjects performed four controlled and four uncontrolled trials using a randomized block design. During the controlled trials, subjects were asked not to smoke or do any vigorous exercise for 24 h, consume a standard dinner provided to them, and to fast for a set length of time ($\pm$ 15 min). During the uncontrolled trials, subjects were asked not to smoke on the morning of the test or to do any unusually vigorous physical activity for 24 h before the test. Subjects were allowed to do usual vigorous physical activity, and were asked to eat their normal dinner meal and to fast between 10 and 14 h. To reduce anxiety, subjects who were not familiar with finger-prick blood glucose response testing conducted a trial test to familiarize themselves with procedure before starting the study. The objective of the study was to see if the difference in subject preparation, controlled vs uncontrolled, influenced the within-individual variation of glycaemic responses expressed as CV (100 × SD/mean). To our surprise, mean CV for the controlled trials (CVc), 24.3 $\pm$ 3.0%, tended to be *greater* than that for the uncontrolled trials (CVu), 20.4 $\pm$ 1.8%, but the difference was not statistically significant. However, CVc was greater than CVu in 10 of the 13 individual subjects (P = 0.046) (Campbell *et al.*, 2003) (Fig. 2.19). The P-value is the chance CVc > CVu on 10 of 13 subjects, assuming that the probability of CVc > CVu is really 0.5. Based on this statistical analysis, we could not conclude that CVc was different from CVu, but we feel this is strong evidence that CVc is not less than CVu.

Thus, this study suggests that controlling subjects' diet and activities the day before a test by the methods we used does not improve the reproducibility of glycaemic response tests, and may actually make it worse. Providing a standard meal and documenting the compliance to the control measures increases the cost of doing studies. Not allowing subjects to do their regular exercise nor eat their regular diet and making them control the fasting time increases the inconvenience to subjects and may make some unwilling to participate in glycaemic response tests. Therefore, until there is evidence to the contrary, I recommend allowing subjects to undertake their usual physical activities and eat their normal diet on the day before doing a glycaemic response test.

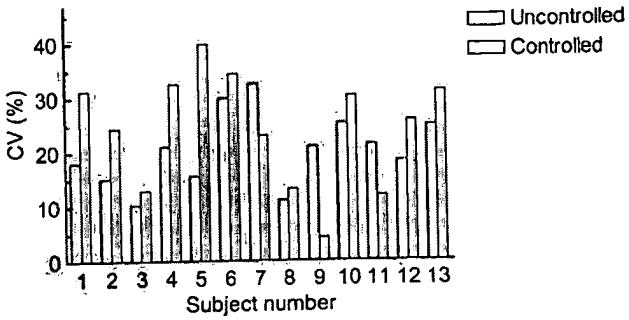

Fig. 2.19. Within-individual coefficients of variation (CV = 100 × sd/mean) of glycaemic responses elicited by 50 g available carbohydrate portions of white bread taken on four occasions after controlled diet and activities the day before or on four occasions after uncontrolled diet and activities in 13 normal subjects. Ten of the 13 subjects have higher CV after controlled diet and activities (P = 0.046) (Campbell *et al.*, 2003).

2.2.8 Effect of volume and type of drink consumed with the test meal

Questions often asked about GI methodology is whether the volume of the test meal should be constant, and what type of drink, if any, can be used.

2.2.8.1 Volume

The volume of test meals influences the rate of gastric emptying (Hunt *et al.*, 1985), which, in turn, affects glycaemic responses (Thompson *et al.*, 1982; Torsdottir *et al.*, 1984, 1986; Mourot *et al.*, 1988; Thomsen *et al.*, 1994). The volume of a test meal can be increased in many ways, for example, by consuming more of the test meal (increased carbohydrate content), or by diluting the test meal with water (reduced osmolarity), or by adding dietary fibre, or even by adding air. The effects of these different manoeuvres on gastric emptying are probably different. However, when considering the volume of a drink consumed with the test meal, the major variables to consider would be the effects of the increased volume and the effects of the reduced osmolarity. Brouns *et al.* (1995) showed that, with a constant delivery of carbohydrate of about 1.2 g/min in various solutions containing 45–90 g/l carbohydrate with osmolarity of 243–374 mOsm/kg, the rate of gastric emptying was not affected by differences in osmolarity, but appeared to be triggered by the carbohydrate content of the drinks. However, the osmolarity of the solutions in this study are much lower than in many nutrition studies in which the effect of volume has been examined (Table 2.9).

In general, several-fold increases in the volume of test meals tend to have a modest effect to increase glycaemic responses, but this is not a consistent finding (Table 2.9). The lack of consistency may relate to the fact that methodology used to test the effect of volume varies widely. Perhaps one of the most important variables is the nature of the carbohydrate in the meal, particularly whether it is soluble, i.e. sugars dissolved in water, or readily hydrated (e.g. cooked potato starch), as opposed to relatively insoluble form, i.e. solid test meal to which water is added.

Schwartz *et al.* (1994) studied the effect of increasing the volume of a 50 g glucose solution from 150 to 450 ml in 132 pregnant women. In the 107 normal women, increasing the volume increased the rise in plasma glucose at 30 min by 12%, but had no effect at 60 min (+0.5%, ns). In the 25 women with gestational diabetes, increasing the volume significantly increased the rise in plasma glucose at 30 min by 17% and at 60 min by 11%. Vuksan's group in Toronto has done a series of studies on the effect on glucose responses of diluting solutions of sugars. In the first study (Sievenpiper *et al.*, 1998), increasing the volume of solutions containing 25 g glucose, sucrose or fructose from 200 to 600 ml significantly increased the incremental area under the finger-prick capillary blood glucose response curve by 30–60% in normal subjects. The results of the second study (Sievenpiper *et al.*, 2000) were consistent with those of the first in showing that as the volume of water in which 75 g glucose was dissolved was increased from 300 to 600 to 900 ml, the area under the finger-prick capillary blood glucose response curve in normal subjects increased by 20% for every doubling of the volume. Each of the ten subjects repeated each volume three times, and the reproducibility of the test was not affected by the volume. The results of the third study differed

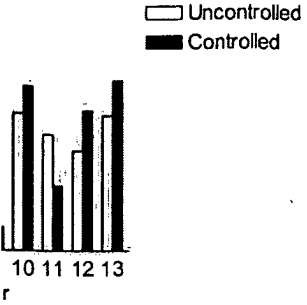

Table 2.9. Effect of volume of test meal on glycaemic responses.

Volume (ml)	Test meal	Osmolarity (mOsm/l)	Subjects (*n*)	% Change in AUC	Reference[a]
150 → 450	50 g glucose	1852 → 617	N preg (107)	+9	1
150 → 450	50 g glucose	1852 → 617	GDM (25)	+14	1
200 → 600	25 g glucose	694 → 232	Normal (8)	+27	2
200 → 600	25 g sucrose	366 → 122	Normal (8)	+33	2
200 → 600	25 g fructose	694 → 232	Normal (8)	+67	3
300 → 600	75 g glucose	1389 → 694	Normal (10)[b]	+18	3
300 → 900	75 g glucose	1389 → 463	Normal (10)[b]	+44	3
300 → 600	75 g glucose	1389 → 694	Lean (11)[b]	−18[c]	4
300 → 900	75 g glucose	1389 → 463	Lean (11)[b]	−37[c]	4
300 → 600	75 g glucose	1389 → 694	Normal (12)[b]	−27[c]	4
300 → 900	75 g glucose	1389 → 463	Normal (12)[b]	−13[c]	4
300 → 600	75 g glucose	1389 → 694	Obese (12)[b]	−3[c]	4
300 → 900	75 g glucose	1389 → 463	Obese (12)[b]	−3[c]	4
0 → 300	Potato/meat	Na	Normal (7)	+68	5
0 → 300	Potato/meat	Na	T2DM (20)	+40	5
90 → 600	Bread/butter	Na	T2DM (10)	−12	6
50 → 250	Test wafer	Na	Normal (12)[d]	+11	7
50 → 500	Test wafer	Na	Normal (12)[d]	+13	7
50 → 750	Test wafer	Na	Normal (12)[d]	+12	7
50 → 1000	Test wafer	Na	Normal (12)[d]	0	7

[a]1. Schwartz *et al.* (1994); 2. Sievenpiper *et al.* (1998); 3. Sievenpiper *et al.* (2000); 4. Sievenpiper *et al.* (2001); 5. Torsdottir and Andersson (1989); 6. Gregerson *et al.* (1990); 7. Young and Wolever (1998).
[b]Each subject repeated each test three times.
[c]Difference in increment at 45 min.
[d]Each subject repeated each test two times.

responses, but this is not a able 2.9). The lack of consist- e fact that methodology used olume varies widely. Perhaps ortant variables is the nature e in the meal, particularly le, i.e. sugars dissolved in ydrated (e.g. cooked potato to relatively insoluble form, which water is added.

(1994) studied the effect of ie of a 50 g glucose solution in 132 pregnant women. In men, increasing the volume plasma glucose at 30 min by ct at 60 min (+0.5%, ns). In gestational diabetes, increas- ficantly increased the rise in min by 17% and at 60 min roup in Toronto has done a ie effect on glucose responses of sugars. In the first study 98), increasing the volume of 25 g glucose, sucrose or fruc- 00 ml significantly increased under the finger-prick capil- sponse curve by 30–60% in e results of the second study 2000) were consistent with showing that as the volume g glucose was dissolved was to 600 to 900 ml, the area k capillary blood glucose re- al subjects increased by 20% the volume. Each of the ten ch volume three times, and the test was not affected by lts of the third study differed from those of the first two in showing that increasing the volume of 75 g glucose solutions from 300 to 900 ml significantly *decreased* postprandial glucose responses in lean, athletic subjects ($n = 11$) and normal lean subjects ($n = 12$) and had no effect in obese normal subjects ($n = 12$) (Sievenpiper *et al.*, 2001). Although, in this last study, glucose was measured in venous plasma as opposed to finger-prick capillary blood, it is difficult to see how this could explain the completely different results.

Torsdottir and Andersson (1989) studied the effect of adding 300 ml water to a test meal of meat and potatoes in normal subjects and subjects with type 2 diabetes divided into those who were well controlled or poorly controlled. Water had the marked effect of increasing the area under the glycaemic response curve in normal subjects by 65%, and in subjects with well-controlled diabetes by 40%. There was no effect of water in subjects with poorly controlled diabetes. The results of this study do not agree with those of another in which either 90 or 600 ml water was added to a meal of rye bread, butter and tomatoes taken by ten subjects with type 2 diabetes (Gregerson *et al.*, 1990). Here, the increase in volume tended, if anything to reduce the area under the glucose response curve by 12%, but the difference was not statistically significant. These results are similar to ours (Young and Wolever, 1998) in which we studied the effect of consuming 50, 250, 500, 750 or 1000 ml water with a solid test meal bar in 12 normal subjects. We found that volume significantly affected the shape of the glucose response curve, but not the IAUC (Fig. 2.20). Increasing volume from 50 to 500 ml tended to increase the area under the glycaemic response curve by 13% (not significant), but the glycaemic response area after 1000 ml water was exactly the same as that after 50 ml. This suggests that the effect of changing volume on glycaemic responses is not linear; as volume increased from 50 to 500 ml, blood glucose at 30 min (peak) increased, and blood glucose at 120 min decreased; but these effects began to reverse as volume continued to increase from 500 to 1000 ml (Fig. 2.21).

Taken together, the above results suggest that large changes in the volume of drink taken with a

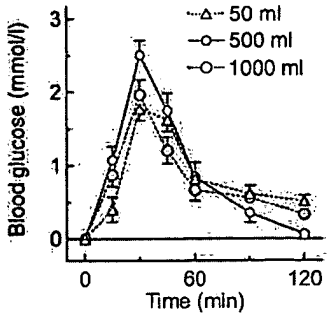

Fig. 2.20. Mean ± SEM blood glucose responses of normal subjects after consuming a standardized solid mixed meal in the form of a wafer with 50, 500 or 1000 ml water. Data from Young and Wolever (1998).

test meal influence glycaemic responses, probably through effects on gastric emptying. This provides a rationale for providing subjects with a drink of a standard volume. If test meals to be compared have large differences in volume, it may be worthwhile controlling for the volume difference.

2.2.8.2 *Type of drink*

Coffee and tea are popular drinks and for many subjects, a drink of hot tea or coffee with the test meal may make the experience of participating as a subject in glycaemic response tests more pleasant than if they were allowed to drink only water. Coffee and tea contain caffeine and related compounds which have many effects on the human body (Donovan and DeVane, 2001; Armstrong, 2002; Heaney, 2002; Smith, 2002; Nawrot *et al.*, 2003). Of particular relevance to the testing of glycaemic responses is the fact that caffeine acutely decreases insulin sensitivity in humans (Graham *et al.*, 2001; Keijzers *et al.*, 2002). Graham *et al.* (2001) gave subjects 5 mg/kg caffeine (average of 385 mg, range 317–468 mg) 1 h before a 75 g oral glucose load, and found a significantly increased plasma insulin response after caffeine compared to placebo. Keijzers *et al.* (2002) administered caffeine intravenously with a 3 mg/kg loading dose followed by continuous infusion at a rate of 0.6 mg/kg/h for 2 h for a total dose of 4.2 mg/kg. Compared to placebo, caffeine impaired whole-body insulin sensitivity, measured with a euglycaemic, hyperinsulinaemic clamp, by about 15% ($P < 0.05$). Both studies demonstrated significant counterregulatory effects of caffeine with significant increases in plasma free-fatty acids (FFAs) and epinephrine.

However, the results of these studies may not necessarily be relevant to the question of whether a drink of coffee or tea should be allowed when determining the GI of foods. The amount of caffeine in tea and coffee varies depending on the nature of preparation, but instant or brewed coffee contains about 65–100 mg per 250 ml cup and brewed black tea 50 mg per 250 ml cup (US Department of Agriculture, 2004). These amounts are only about 15–35% of those used in the caffeine studies cited above. In addition, coffee and tea contain more than just caffeine, and the methods of administration used in these studies may not reflect the pharmacokinetics of caffeine obtained from a drink of coffee or tea taken with a test meal. Thus, it would seem more instructive to look at the effects of coffee or tea on postprandial responses.

Coffee and tea stimulate gastric secretions (Dubey *et al.*, 1984; Coffey *et al.*, 1986; Boekema *et al.*, 1999), reduce glucose-dependent insulinotropic polypeptide (GIP), increase glucagon-like peptide 1 (GLP-1) secretion (Johnston *et al.*, 2003) and may accelerate gastric emptying (Lien *et al.*, 1995). When we compared the effect taking 250 ml water, coffee or tea with a standardized solid test meal bar, we found that blood glucose at 45 min was significantly higher after coffee and

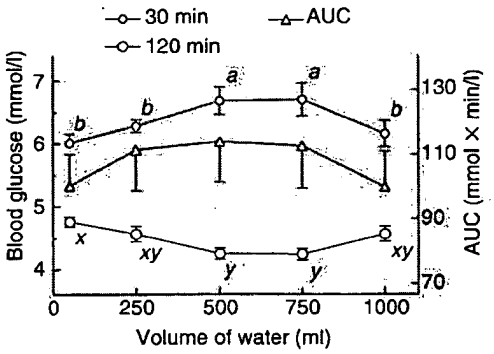

Fig. 2.21. Effect of consuming different volumes of water with a standardized solid test meal on blood glucose concentrations at 30 and 120 min and incremental area under the blood glucose response curve in normal subjects. Values are mean ± SEM. Means with different letters differ significantly ($P < 0.05$). Data from Young and Wolever (1998).

tea compared to water, but there was no significant effect on the incremental area under the glycaemic response curve (Fig. 2.22; Young and Wolever, 1998).

These results are similar to those from Johnston *et al.* (2003) who found significantly higher plasma glucose 30 min after coffee containing 25 g glucose, compared to 25 g glucose dissolved in water, but no significant effect on the incremental area under the glycaemic response curve.

In terms of determining the GI of foods, the question is not so much whether coffee and tea influence glycaemic responses, but whether they influence either the mean or the variability of the GI. Unfortunately, there are no data addressing this issue. However, if coffee had the same relative effect on postprandial glycaemic responses of all test meals, it would have no effect on the calculated GI value, because the AUC of both the test food and the reference food would be increased to the same extent: i.e. $A/B = kA/kB$, where A and B are the AUC values for the test and reference foods, respectively, and k is the effect of coffee on AUC. It is possible that the effect of coffee and tea on postprandial responses may differ for different carbohydrate sources, but this has not been investigated. Coffee and tea might conceivably influence the variation of glycaemic responses by stimulating gastric activity and making subjects feel better, but data here are also lacking.

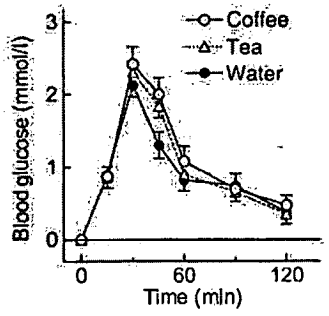

Fig. 2.22. Effect of consuming 250 ml coffee or tea compared to 250 ml water with a standardized solid test meal bar on blood glucose responses in normal subjects. Blood glucose at 45 min was significantly higher after coffee and tea than water, but there was no significant effect on incremental area under the curve (Young and Wolever, 1998).

2.2.9 Time to consume test meal

It is known that consuming glucose (Jenkins *et al.*, 1990), liquid test meals (Wolever, 1990a) or solid test meals (Jenkins *et al.*, 1982b, 1992; Bertelsen *et al.*, 1993; Segura *et al.*, 1995) in small divided doses over a period of 3–4 h has a marked effect in flattening the glycaemic response compared to consuming exactly the same test meal over 10–15 min. However, the effect of varying the time of ingestion of solid meals over periods of less than 30 min has not been investigated. Heine *et al.* (1983) showed in healthy volunteers that consuming 75 g glucose or hydrolysed starch in 300 ml water over 1 min, as compared to 10 min, reduced the netAUC by about 50%. For these reasons, it appears appropriate to recommend that the test meal is consumed in about 10–15 min, depending on the type of food.

2.3 Conclusions

If the GI is to become a useful marker of the biological effects of carbohydrate foods, the first thing we must be clear about is what it means. Nutrition scientists and professionals must appreciate the difference between the 'glycaemic index' and a 'glycaemic response'. The GI is determined by measuring the glycaemic responses elicited by feeding reference and test foods to human subjects on separate occasions. There are many ways to measure glycaemic responses, and methodological differences may or may not influence the results. Factors, which affect the glycaemic responses will, therefore, affect the GI values obtained. If the GI is to be practically useful, it must be a function of the carbohydrate food which can be measured reproducibly. Thus, standardized methodology is required. However, this does not necessarily mean that every experimental variable must be rigidly controlled. This could lead to methods which are so difficult and restrictive that they would render the GI practically useless because it was too expensive to measure. Some things which might seem to be very important to control (e.g. the type of subject studied) may have little impact on the results obtained or may actually tend to make the results worse (e.g. standardized meal the night before). Some things which might not even be considered (e.g. the type of blood sampling) may

actually be quite important to the results obtained. Thus, we need to understand the sources of error in the methodologies used and how these errors affect the results obtained. Methods can then be developed with an appropriate balance of cost vs precision. There are many more questions about methodology for which we have no answers than there are questions about which we have some information. However, since studies on methodological factors have often produced surprising results, I would strongly recommend that changes to GI methodology should not be translated into practice until the effects of such changes on the results obtained have been investigated, particularly for changes which make it more expensive or difficult to carry out GI tests.